Chapter 2

Serial reversal learning and acute tryptophan depletion

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Abstract

Cognitive flexibility (i.e. the ability to adapt goal-directed behaviour in response to changed environmental demands) has repeatedly been shown to depend on the prefrontal cortex (PFC). Recent data from primate studies moreover show that depletion of prefrontal 5-HT impairs reversal learning of visual stimuli (Clarke et al., 2005, 2007)

It is not clear however if 5-HT serves a general role in reversal learning or if it is involved only in specific reversal problems. A first aim of these experiments was to study the role of 5-HT in serial reversal learning of a spatial discrimination. Literature has, moreover, repeatedly shown that the PFC is involved in the initial acquisition of a reversal problem but hardly when the task is well practiced. A second aim concerns the role of 5-HT in early versus late reversal learning. With the current experiment, we aim to clarify whether 5-HT is differentially involved in early versus late reversal learning. To this end, we tested rats on a serial two-lever reversal task and induced a temporary reduction of 5-HT availability in these rats by restricting dietary intake of the 5-HT precursor tryptophan at an early and a late reversal. Our results indicate that acute tryptophan depletion (ATD) did not affect either early or late reversal learning, nor extinction and suggest that spatial reversal learning, in contrast to visual reversal learning, might not be dependent on 5-HT. The data furthermore provide insight in the behavioural strategies employed in serial reversal learning and suggests the formation of a learning-set.

Introduction

Cognitive flexibility, or the ability to adapt ongoing behaviour to environmental changes, depends critically on the prefrontal cortex (PFC) and its connection with thalamic and striatal areas (e.g. Block et al., 2007). Functions that underlie this ability, moreover, like response selection, inhibition and extinction, as well as encoding of reward-

related information have been shown to be impaired after lesioning or inactivation of prefrontal cortex (PFC) areas (for a review, see Dalley et al., 2004; Robbins, 2005).

In studies of cognitive flexibility, reversal learning is a commonly used paradigm in which a previously learned stimulus – reinforcement or action – outcome association is reversed and subjects have to adjust their behaviour accordingly. Performance on various behavioural tasks based on this paradigm have been shown to depend on the PFC in both primates (Bechara et al., 2000; Cools et al., 2002; Hornak et al, 2004; Manes et al., 2002; O'Doherty et al., 2001) and rats (Chudasama and Robbins, 2003; De Bruin et al., 2000; Joel et al., 1997; Li and Shao, 1998; McAlonan and Brown, 2003; Salazar et al., 2004, Schoenbaum et al., 2002; Wishaw, 1985).

Previous work stressed the importance of mono-aminergic innervation of the PFC in cognitive flexibility in general (e.g. Bouret and Sara, 2005; Lapiz and Morilak, 2006; Robbins, 2000) and reversal learning in particular (Masaki et al., 2006; van der Meulen et al., 2003). Evidence for a specific role of *prefrontal* 5-HT is suggested by work of Clarke and collaborators (Clarke et al., 2004, 2005, 2007) in primates. It is not clear however if 5-HT serves a general role in reversal learning or if its involvement depends on the particular stimulus modality. Previously we developed a reversal task (De Bruin et al., 2000) with which we showed medial PFC dopamine (DA) involvement in reversal learning of a spatial discrimination. A decreased performance on the first reversal was reported by De Bruin et al. (2000) after local application of a D1 receptor antagonist, while a subsequent microdialysis study showed increased efflux of DA during execution of this task (van der Meulen et al., 2007). In view of current literature that implicates serotonin (5-HT) as an important neurotransmitter in (primate) reversal learning of visual stimuli (Clarke et al., 2005, 2007), the exploration of the role of 5-HT in spatial reversal learning is the first aim of the current study.

The role of the PFC, moreover, seems particularly important for reversal learning during the early stages when the task is still new but not when the task is well trained. Functional MRI studies in humans show that, in general, activation of the PFC lessens as learning progresses (Passingham, 1998, Petersen et al., 1998; Raichle et al., 1994), suggestive of reduced PFC input after initial learning. Similar observations were made in rats by De Bruin et al. (2000), who showed that inactivation of the medial PFC resulted in impaired reversal learning only in early reversals, but not during subsequent reversals of the same stimuli (serial reversals) (see also, Divac, 1971; Nonnemen et al., 1974; van der Meulen et al., 2003, 2007). This reduced PFC involvement after repeated testing is possibly related to the formation of a learning-set, which allows for rapid acquisition of subsequent learning problems (Harlow, 1949). In line with the experiments of Raichle et al. (1994), Yokoyama et al. (2005) showed learning-set formation related changes in PFC activation. The evidence for diminished PFC involvement over training is however, inconclusive, as electrophysiological recording of PFC neurons in primates and rats

show sustained activation throughout testing (Schoenbaum et al., 2006; Thorpe et al., 1983) and Kolb et al. (1974) reported effects of medial PFC lesions on 'late' reversal learning. Involvement of 5-HT in repeated reversal learning, moreover, has, to the best of our knowledge, not been investigated.

The method of acute tryptophan depletion (ATD) was employed in the current study to study the effect of transient 5-HT depletion on reversal learning, both early in training and after repeated testing when reversals were rapidly acquired. During serial reversal learning of a spatial discrimination, animals were treated with either ATD or a control diet.

Materials and methods

All experiments were approved by the Animal Experimentation Committee of the Royal Netherlands Academy of Arts and Sciences and were carried out in agreement with Dutch Laws (Wet op de Dierproeven, 1996) and European regulations (Guideline 86/609/EEC).

Subjects

Subjects were male outbred Wistar rats (Harlan/CPB, Horst, The Netherlands), weighing 175–200 g at arrival (8 for experiment 1; 16 for experiment 2). The animals were socially housed in groups of four in standard type IV macrolon cages where they were kept under a reversed day/night cycle (dimmed red light from 7 a.m. until 7 p.m., white light from 7 p.m. untill 7 a.m.) for the duration of the experiment. Throughout the experiment, the animals were food-restricted (16 g/animal/day) to maintain their body weight at 90% of free-feeding weight.

Apparatus

The experiments were performed in locally constructed operant chambers that were equipped with a set of two retractable levers positioned to the left and right of a food dispenser. A house light was placed on the opposite wall, two lights were positioned above the levers, signalling their presentation, and one inside the food dispenser, signalling the delivery of a 45 mg food pellet (Noyes Formula P, Research Diets, NewBrunswick, NJ, USA). Nose-pokes into the food well were detected by an infrared sensor located inside the food well. Operation of the Skinner boxes was controlled by a pc running Med-pcTM software (Med Associates, Sanddown Scientific, Middlesex, UK).

Experimental procedure

Approximately 2 weeks after arrival, the test groups were created with the body weight of the animals matched between groups. Four groups were formed, two for a control

experiment that assessed the effect of treatment on general activity (experiment 1) and two groups that were tested on the serial reversal test (experiment 2). On experimental days, which typically lasted from 9 a.m. to 5 p.m., the animals were transferred to the experimental room where they remained for the entire day. In both the animal facility and the experimental room, a radio played to mask background noises.

Acute tryptophan depletion (ATD) or a control diet was given once in experiment 1 (see below) and four times during experiment 2 (during 2 reversal sessions and 2 extinction sessions, see below).

The procedure for days on which behavioural testing was combined with ATD, differed only from 'non-ATD' days in that the animals were treated with either a diet lacking tryptophan (TRP) or a control diet with added tryptophan [procedure as described in Lieben et al., 2004b]. The two groups from experiment 2 functioned alternately as control and TRP group, the animals that were assigned to experiment 1 were only tested once. Three hours after the initial diet administration (see below, *treatment*) the behavioural testing started. On days without ATD, the animals remained a similar period of time in the experimental room prior to behavioural testing.

Experiment 1; general activity (FIFR, control experiment)

To assess the possible effect of ATD on general activity, a separate group of 8 animals was tested on a fixed interval, fixed ratio (FIFR) task. For this task, the animals were trained to press a lever at least three times (FR3) in a 40-second time window (FI40) to obtain a food reward. A reward was given at the end of the interval only if the FR3 was reached. Although the levers were presented throughout the session, additional lever pressing within the 40-second window was not rewarded. After a steady level of lever pressing had been obtained, the animals were subjected to a single session with either ATD or treatment with the control diet, as described below.

Experiment 2; two-lever spatial discrimination and reversal task

This task was used to assess the effects of ATD on the early, and late reversal learning in a series of reversals of a two-lever discrimination and the extinction of the learned response.

First, the animals were trained, in a maximum of six (40 trial) shaping sessions, to press a lever for a food reward. During this shaping phase, a single lever is presented at each trial (randomly the left or right lever). In the course of the sessions, the animals are required to increase the number of lever responses per trial from a fixed ratio (FR) of 1–3 responses to obtain a reward. All of the trials in the two-lever spatial discrimination and reversal task (including shaping) were discrete choice trials, i.e. the lever(s) were retracted upon responding or, in case of an omission, at the end of the trial duration (60 sec). The intertribal interval was set at 25 seconds.

After the animals had completed the initial shaping (i.e. respond to the lever for a food reward) a second lever was introduced and the animal learned to press either the left or right lever to obtain a food reward (two-lever discrimination). Pressing the incorrect lever or omitting a response leads to trial termination and a 25-second inter-trial interval time-out. After reaching criterion on the two-lever discrimination, i.e. obtaining 95% of the food pellets in a (64 trial) session, the animals were subjected to serial reversals. During this reversal phase the rewarded and non-rewarded levers were reversed daily for 18 days. The final phase was an extinction phase in which both levers were presented but none are rewarded.

For both the two-lever discrimination and the serial reversal tests goes that the animals were tested twice daily: a morning and an afternoon session. Animals that did not reach a 95% performance criterion during reversal learning (on any of the eight-trial blocks, and on each reversal day) were excluded from the experiment. The extinction phase consisted of a single daily session for four consecutive days. All sessions comprised eight blocks (with eight trials each) which were divided for analysis into two sets of four blocks. Session duration ranged from 45 min (two-lever discrimination) to 2 h (extinction).

The ATD treatment was given twice during the reversal phase (reversal 1 and 16) and twice during the extinction phase (session 1 and 3). No treatment was given during the stages preceding the reversal phase.

Treatment

The experimental group (TRP group) was treated orally with a protein–carbohydrate mixture, lacking tryptophan (TRP) (4.0 g/kg Solugel DTM and 2.0 g/kg Maltodextrine) in a volume of 10 ml/kg. Two diet administrations were given, spaced 90 min apart. Control animals received the same mixture with added TRP (0.28% TRP of total protein). The procedure for administration is identical to that described in Lieben et al. (2004b). To minimize stress the rats were daily handled and habituated to oral injections with normal tap water (10 ml/kg). To minimize TRP intake through the normal rat chow, food was removed from the home cages 14 h before the start of the experiment. Water, however, remained freely available during this period.

Drugs and chemicals

The Gelatine hydrolysate (Solugel DTM) was obtained from PB Gelatins (Tessenderlo, Belgium). Glucodry 200 was obtained from the Amylumgroup (Koog aan de Zaan, the Netherlands). Tryptophan was obtained from Sigma–Aldrich Chemie, Germany.

Behavioural measures

Experiment 1; general activity (FIFR, control experiment)

To assess the possible effects of ATD on general activity and motivation for lever pressing, the TRP group and control group were compared regarding average number of head entries into the food dispenser and average number of lever presses. To exclude the influence of previous performance on these activity measures, data from the treatment day were compared to the final acquisition session on the day before (both sets of four blocks). Performance on this task was measured as the percentage of pellets that was obtained.

Experiment 2; two-lever spatial discrimination and reversal task

The data obtained from the behavioural task were taken as measures of performance in the following fashion: both the amount of rewards as a fraction of the amount of *obtainable* rewards and the number of correct and incorrect lever responses were taken as an index of overall performance on all test phases except the extinction phase of the two-lever discrimination. For this phase, the total number of lever responses was taken as the index. And, as this measure is prone to interference from pre-existing differences between groups in the total number of lever presses, the amount of lever presses per block of eight trials was expressed as a percentage of the mean number of lever presses per group in the same block on the last reversal day. Omissions were scored on both (reversal) ATD treatment days to assess the effect of treatment on this parameter.

To assess learning over serial reversals, responses on the correct lever were scored as a percentage of the total number of lever responses. The measure of percentage responses correct, as opposed to percentage pellets obtained was chosen to exclude interference from omissions. The number of animals that made no errors (i.e. responses on the incorrect lever) in the first block (eight trials) of each session was counted and scored as the number 'error-free' reversals per session. Lever preference (left versus right) was assessed by comparing the cumulative number of lever presses on each lever during extinction learning when both levers were present but not rewarded.

Data analysis

Behavioural data taken during these experiments were analyzed using SPSS for Windows (Version 11.0; SPSS, Gorinchem, Netherlands). Based on previous results, in which behavioural effects were often observed only in one half of a learning session, we analysed the data per session but also per set of four blocks, each of which consisted in turn of eight trials (De Bruin et al., 2000). A repeated measures ANOVA was used with treatment (control/TRP group) as 'between-subjects' factors and time as

'within subjects' factor. When a treatment or interaction effect was found, a one-way ANOVA and independent-samples *t* test was used to test for group differences and time effects within groups. FIFR data were analyzed similar to the data of the two-lever discrimination task. In case an effect of ATD was found, a covariate analysis was performed to exclude the possibility that ATD effects were due to pre-existing group differences. To examine learning and responding strategy over the 18 subsequent reversals, the data of both groups were pooled and analyzed with a repeated measures ANOVA with repeated contrast, comparing performance over the first set of every day (32 trials) to the previous day (De Bruin et al., 2000). Pooled data were also analyzed in a similar fashion for the extinction sessions, with respect to the amount of lever presses on the left or right lever.

For each ANOVA the number of degrees of freedom was adjusted by a Huyn-Feldt correction when indicated by Mauchly's test of sphericity. For the repeated contrasts, a Bonferroni correction was applied.

Results

Experiment 1; general activity (FIFR, control experiment)

The effect of ATD on general activity was analysed by comparing the amount of lever presses and nose-pokes between treatment-groups on the FIFR test. Figure 1 shows the average number of lever presses and nose-pokes for the final acquisition session and the ATD test session. Data analysis of the final acquisition day revealed no group, or interaction effects for either measure. A time effect for nose-pokes ($F(7,42)=2.296$, $p=0.045$), but not lever presses was found over all blocks of this session.

Analysis of the ATD session revealed no effect of the treatment on nose-poke activity, nor did we observe a time or interaction effect. An ANOVA with repeated measures over the lever press activity revealed no time effect over either set of four blocks. A main effect of group, however, was observed when both sets were taken together ($F(1,6)=7.102$, $p=0.037$). Similarly an interaction effect was found ($F(7,42)=2.896$, $p=0.015$). An independent samples *t* test over this session showed blocks 4, 5 and 7 to differ between groups, respectively ($t=4.445$; $p=0.010$), ($t=3.755$; $p=0.024$) and ($t=4.948$; $p=0.003$). A covariate analysis revealed that the effects found during the ATD test session could not be attributed to pre-existing group differences.

No effect of ATD was observed on performance, both groups obtained the maximum amount of pellets during this task (data not shown).

Experiment 2; two-lever spatial discrimination and reversal task

Shaping and two-lever discrimination

During the initial shaping phase, in which the animals learned to press a lever for a food reward, no differences between the groups were observed on any of the behavioural measures. Shaping took six sessions spread out over 7 days. Performance, expressed as a percentage of obtained rewards, exceeded 90% prior to initiation of the next test phase (data not shown).

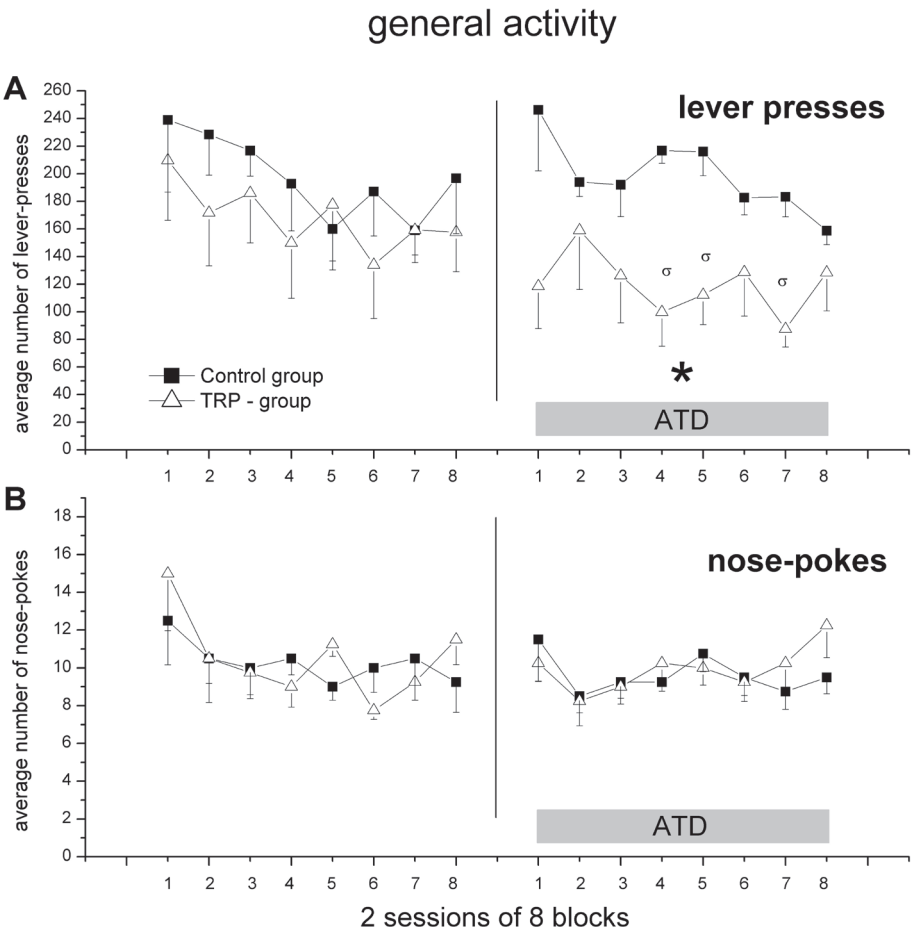

Fig. 1. Experiment 1: general activity in the FIFR-task. (A) The average number of lever presses (mean±S.E.M.) for the TRP group and control group and (B) the average number of nose-pokes (mean±S.E.M.) for both groups are depicted for the final acquisition session (left) and in the subsequent session following ATD (right). * indicates a group difference $p<0.05$. σ indicates differences between independent samples $p<0.05$.

Shaping was followed by the two-lever discrimination phase in which one out of two available levers was rewarded (five morning sessions). A main effect of block, showing that learning took place, was found over both sets of the first session ($F(7,98)=24.197$, $p=0.000$) and second session ($F(7,98)=4.614$, $p=0.000$) (data not shown). No group or interaction effects were observed during the acquisition sessions. Performance measures in the final acquisition session showed that both groups obtained the maximum amount of pellets (Fig. 2A) and did not make incorrect responses (Fig. 2B).

Spatial reversal

After reaching criterion on the two-lever discrimination, the animals were subjected to 18 serial reversals. ATD was administered before the first and 16th reversal. The results of the first reversal are shown in figure 2. Each animal reached the 95% performance criterion at the end of this, and all subsequent reversal sessions (data not shown). A main effect of block, for the percentage pellets that were obtained, was observed for each of the morning sessions, throughout all reversals. After the tenth reversal session, no block effects were observed for the afternoon sessions. Similar to De Bruin et al. (2000) we analyzed the percentage of correct responses over the first 32 trials (first set) of each morning session. Figure 3 shows that learning continued throughout the 18 reversals ($F(17,238)=133.132$, $p=0.000$), and performance continued to improve. A subsequent repeated contrast analysis over combined data, moreover, revealed that certain sets were significantly different ($F(1,15)$ between 74.578 and 12.564; $p\leq0.03$, alpha Bonferroni corrected) from the previous set (Fig. 3). A gradual increase in the number of animals that showed 'error-free' reversal learning, coinciding with improved performance, was observed over the serial reversals (Fig. 3).

ATD-reversal 1 (first reversal); A main effect of block (not group or interaction) was observed over both sets of the morning session of the first reversal for the percentage pellets obtained ($F(7,98)=30.458$, $p=0.000$), and the number of correct (and incorrect) lever responses (resp. $F(7,98)=28.554$, $p=0.000$; $F(7,98)=32.238$, $p=0.000$). During the afternoon session a block effect over both sets taken together indicated that learning continued; percentage pellets obtained ($F(7,98)=11.675$, $p=0.000$), correct responses ($F(7,98)=12.195$, $p=0.000$) and incorrect responses ($F(7,98)=15.088$, $p=0.000$). Further analysis revealed no group or interaction effect for any of the performance measures, neither for individual sets nor for complete sessions (Fig. 2A and B). No group or interaction effects were observed for the second reversal session that followed the initial reversal (data not shown). No omissions were made by any of the animals during the first reversal sessions (morning and afternoon).

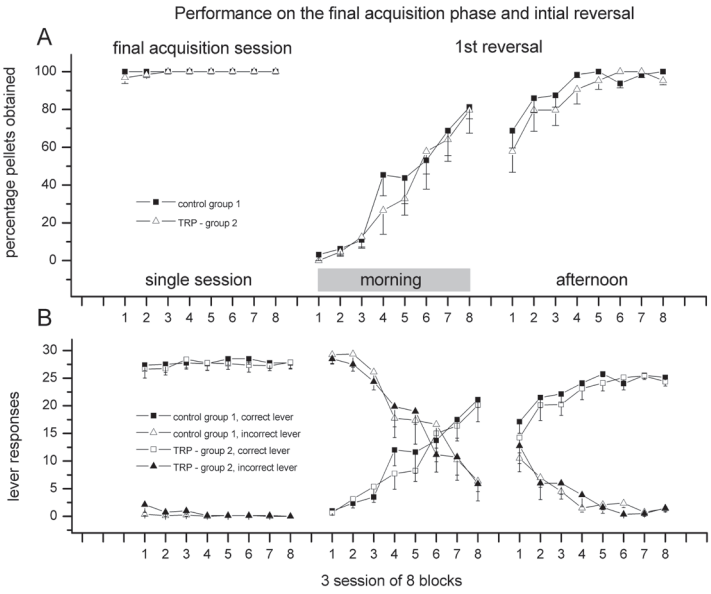

Fig. 2. Experiment 2: performance on the final acquisition session and initial reversal of the serial reversal task. The percentage of pellets (mean±S.E.M.) that was obtained in the final acquisition session (left) and first reversal, when ATD was administered (right) is shown in A. B shows the number of correct and incorrect lever responses (mean±S.E.M.). The grey box indicates the session during which ATD was administered.

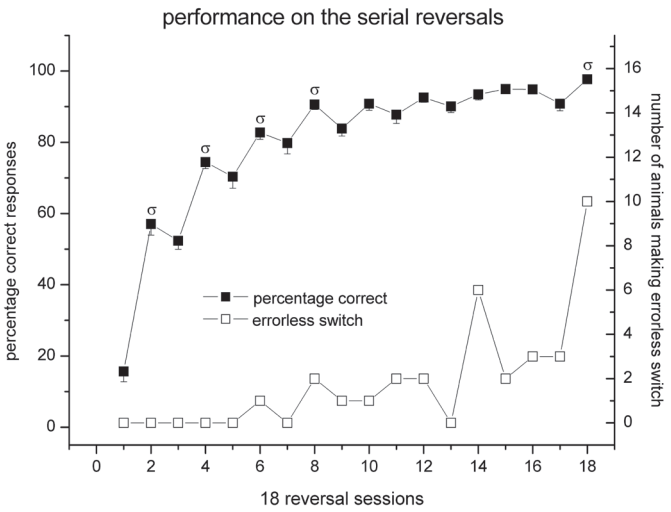

Fig. 3. Performance on the 18 consecutive reversals of the serial reversal task. Depicted is the percentage of correct responses (black filled squares) (mean±S.E.M.) over the first set (four blocks of eight trials) of each day (left *y*-axis) and the number of animals that made no errors during the first block of each day (error-free switching). *σ* indicates a significant difference between the performance of that set compared to the previous set $p \leq 0.05$.

ATD-reversal 2 (16th reversal). After the increase in performance on the reversals had leveled off (i.e. a stable performance of ≥95%) the animals were subjected to a reversal with ATD. A main effect of block (not group or interaction)was observed over both sets of the morning session for percentage pellets obtained ($F(7,98)=2.689$, $p=0.014$), correct responses ($F(7,98)=3.459$, $p=0.020$) and incorrect responses ($F(7,98)=9.160$, $p=0.000$). During the afternoon session no block effects were observed. Further analysis revealed no group or interaction effect, for individual sets or complete sessions for any of the performance measures. No differences were found over the second set of the afternoon session (Fig. 4B). No group or interaction effects were observed for the following, 17th reversal, session data not shown). During the 16th reversal both the control and ATD treated group did make omissions but were not statistically different ($F(1,14)$ between 1.166 and 0.684; $p>0.3$) (data not shown).

Final reversal session

Figure 4B shows performance on the 18th, and last, reversal, for which no group or interaction effects were found. During this session, in which both groups reach near perfect performance, a total of 10 animals (out of 16) made an error-free switch (Fig. 3).

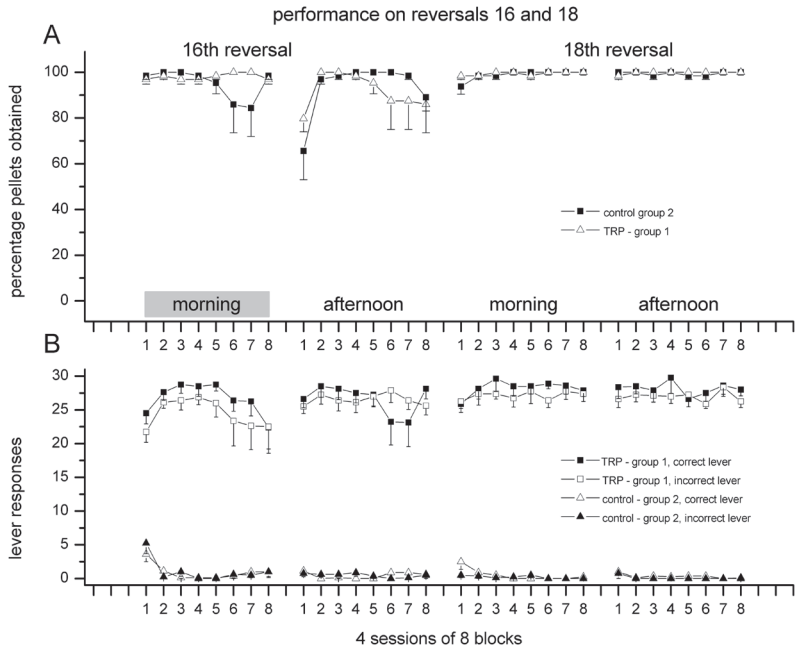

Fig. 4. Performance on the 16th and final reversal session. (A) The percentage of pellets (mean±S.E.M.) that was obtained during the 16th reversal session, when ATD was administered (left) and 18th (final) reversal (right). (B) The number of correct and incorrect lever responses (mean±S.E.M.). The grey box indicates the session during which ATD was administered.

Extinction

After 18 consecutive reversals the animals were subjected to an extinction phase during which lever pressing was no longer rewarded. Alternately, both groups underwent ATD and served as control group (extinction sessions 1 and 3). When comparing the total amount of lever presses corrected for base-line lever pressing, both groups showed a steady decrease over both sets of each of the four sessions (main effect of time: $F(7,98)$ between 59.197 and 18.862; $p=0.000$) (data not shown). There were, however, no main effects of treatment, nor interaction effects on any of the four measuring days. Over the course of 4 days, lever pressing was never totally extinguished. Analysis of the uncorrected extinction data yielded identical results.

An additional analysis over pooled data of both groups revealed that over both sets of the first extinction session (but not over subsequent sessions) the animals responded significantly more to the lever that was initially reinforced during training than the other lever ($F(1,30)=4.963$, $p=0.034$) (Fig. 5).

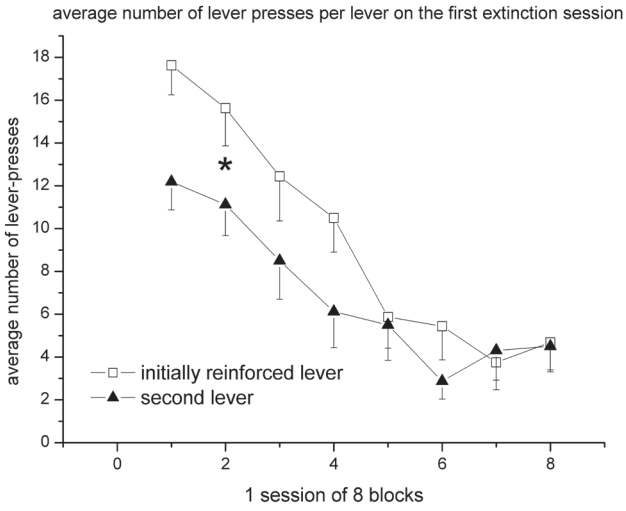

Fig. 5. The average number of lever presses per lever (mean±S.E.M.) over the first extinction session is depicted. * indicates a significant difference ($p<0.05$) in between the initially reinforced lever and the other (initially unrewarded) lever.

Discussion

The current experiment was aimed at clarification of the role of 5-HT in serial reversal learning of a spatial discrimination in the rat. Our results indicate that a transient reduction of 5-HT availability through acute tryptophan depletion (ATD) did not affect performance on the initial reversal, neither when the task was new, nor on the 16th reversal, when the task was well learned.

Lever directed activity, however, was found to be reduced in tryptophan-depleted animals, independent of general activity and in the absence of effects on task performance. The data furthermore indicate that the progressive increase in performance on the serial reversals is due to a decrease in perseverative responding and an increase in error-free switching. ATD did not affect the formation of this learning-set.

The method of ATD we used for the current experiment temporarily reduces 5-HT availability in the brain (Biggio et al., 1974; Fadda, 2000; Fadda et al., 2000b; Gessa et al., 1974), and has been shown to induce behavioural effects across species (Booij et al., 2002; Delgado, 2006; Lieben et al., 2004b; Riedel et al., 2002). However, it should be mentioned that, as we have not measured actual release of 5-HT following ATD, we do not know to what extent 5-HT was depleted and if the magnitude of depletion was sufficient to induce reversal learning impairments. Nevertheless, a transient reduction in the number of lever presses during the 'fixed interval fixed ratio' control experiment (experiment 1) following ATD suggests that the chosen method as well as the time window of behavioural testing was successful in inducing behavioural effects.

Although similar effects on activity measures following 5-HT depletion have not been observed, these results are very similar to those observed by Price et al. (1977) after nor-adrenergic lesions. In their experiment, lesioned animals showed a selective decrease in lever responding on a variable interval task, in the absence of a generalized reduction of activity or decreased task performance. It was suggested by these authors that this behavioural pattern could indicate increased sensitivity to the conditions of reinforcement resulting in greater efficiency.

For the testing of cognitive flexibility we used a reversal learning task that was previously developed in our lab and with which we showed medial PFC involvement in spatial reversal learning (De Bruin et al., 2000). The task posed the animals with a reversal problem; a previously reinforced stimulus (e.g. the left lever) requiring a response (lever press) was no longer rewarded, while the previously unrewarded stimulus (e.g. the right lever) obtained the reinforcing property.

Data showed that the initial stimulus discrimination was quickly acquired and that the animals obtained all rewards by the last (fifth) session and made no incorrect lever responses. The initial reversal, moreover, was learned within the first day (i.e. obtaining >95% of the rewards). After that, performance kept improving over 18 subsequent reversals. Responding decreased when rewards were no longer given during the extinction but never completely seized.

The present experiment was designed to dissociate the possible role of 5-HT in early learning, when reversals were new, from late learning, when reversal learning was well trained. Although no 5-HT involvement was shown in either one (see below), the current experiment revealed the strategy that the animals employed during reversal learning.

Like previous work (De Bruin et al., 2000; van der Meulen et al., 2007; Wishaw, 1985) we showed that over repeated sessions performance kept improving, possibly due to the formation of a learning-set (Fagan and Olton, 1987; Harlow, 1949; Whishaw, 1985).

Interestingly, the current data suggests that this improved performance might not be solely due to a decrease of perseverative errors on the previously rewarded lever (i.e. the lever that was rewarded in the previous session). In contrast to previous work by De Bruin et al. (2000) we found that when rats had to switch 'away' from the initially reinforced lever (i.e. the lever that was rewarded during task acquisition) they performed worse than when they reversed 'back' to the initially rewarded response (Fig. 3). This suggests that rats developed a preference for the initially reinforced lever and showed greater difficulty overcoming perseverative responding when responding to this lever. Despite the fact that this observation was only made during the early reversals and not during the late reversals (suggesting that this type of perseverative errors were overcome in the course of the serial reversals), extinction data showed that when neither lever was rewarded the rats responded significantly more on the lever that was initially rewarded. Together these data show that lever preference developed during intial training is never totally lost, and continues to influence performance even after extensive training and when performance indicates otherwise.

The only apparent difference between the current experiment and that of De Bruin et al. (2000) is the lidocaine treatment that was given in the latter experiment as opposed to the ATD treatment of the current experiment. It is however not clear how this difference might explain the observed pattern of perseverative responding.

Only after repeated testing did the rats gradually reduce overall perseverative errors and start to exhibit error-free reversal learning (Fig. 3) between sessions. The latter observation is remarkable as it suggests that rats were able to retain information of the previous session and use this information to anticipate the correct (changed) stimulus reward combination and act accordingly. The current experiment however, was not designed to assess the effect of ATD on memory consolidation; therefore we cannot confirm the observation of impaired memory function after ATD (Lieben et al., 2004a; Riedel et al., 2002; Rutten et al., 2007).

The absence of an effect of ATD in a medial PFC mediated spatial reversal task adds to the existing literature that suggests a possible dissociation between reversal learning of spatial information on the one hand and visual/odour information on the other.

Serotonin involvement in reversal learning of visual or olfactory discriminations has been shown in relation to orbital PFC functioning in primates (Fagan and Olton, 1987; Rogers et al., 1999a,b, 2003). 5-HT depletion studies moreover, have shown involvement of 5-HT in functions that are related to cognitive flexibility like stimulus reward learning, perseverative responding, response inhibition and memory

consolidation (Beninger and Phillips, 1979; Dalley et al., 2002; Harrison et al., 1999; Rogers et al., 1999a; Soubrié, 1986; Winstanley et al., 2004a, 2006), but also reversal learning in rats (Masaki et al., 2006). These results could suggest that reversal learning is solely dependent on orbital PFC 5-HT. However, Masaki et al. (2006) present correlational evidence that the medial PFC 5-HT might also be involved in reversal learning. Literature moreover, indicates that in contrast to visual and olfactory-based learning, reversal learning of spatial information depends on the medial PFC (De Bruin et al., 2000; Kolb et al., 1974; Li and Shao, 1998; Salazar et al., 2004). The fact that we do not observe ATD induced impairment in the current experiment suggests that the involvement of 5-HT in reversal learning might be modality specific and follows the anatomical separation of visual and olfactory reversal learning in the rat. As was mentioned briefly in the introduction, our lab reported earlier that dopamine efflux in the medial PFC increased during early reversal learning (van der Meulen et al., 2007) and that local infusion into the medial PFC of a D1 receptor antagonist impaired reversal learning one the same task (De Bruin et al., 2000). A recent study of DARPP-32 knock-out mice by Heyser et al. (2000) provide further indications that dopamine is involved in reversal learning of a spatial discrimination. Support for the notion of a possible dissociation between visual/olfactory reversal learning comes from a recent study by Clarke et al. (2007) who showed that DA is *not* involved in reversal learning of visual information.

Further experiments need to be performed to support the present findings and show a full separation of medial PFC mediated spatial reversal learning and orbital mediated visual/olfactory information.

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