The role of the endocannabinoid system in object recognition memory impairments by glucocorticoids and emotional arousal.

By Jelle W. de Boer

Under supervision of:
Dr. Piray Atsak
Prof. Dr. B. Roozendaal (research study)
Prof. Dr. E. A. van der Zee (written report)

Research project report for the Master Biomedical Sciences, RUG department Neuroanatomy, Groningen

31st of August, 2016

TABLE OF CONTENTS

INTRODUCTION

Background research of this study

The group of neuroanatomy in the University Medical Centre Groningen focuses on emotional effects on memory processes. In processing experiences, the brain is influenced by emotional context. A case in point is acute stress. Such an experience activates a cascade of physiological responses, among which the release of hormones. This stress activated cascade is referred to as the hypothalamus-pituitary-adrenal axis. It is well preserved in mammals and culminates in the release of glucocorticoids. Behaviour is immediately affected and this emotional state has a profound effect on memory. Interaction between the amygdala and the hippocampus plays a central role here.(Nathan, Griffith, McReynolds, Hahn, & Roozendaal, 2004; B Roozendaal, 2002; Benno Roozendaal, Okuda, Van der Zee, & McGaugh, 2006)

Advances in memory research have led to a broad understanding of the anatomical and cellular basis for memory processes. They are time dependent and include acquisition, consolidation, retention, retrieval and extinction. The amygdala can modulate some of these processes depending on emotion and arousal. This is part of how the brain gains it adaptability and selectivity. It has been found that glucocorticoid hormones and noradrenergic neurotransmission are required for modulation of memory resulting from acute stress and emotional arousal. The mechanisms underlying this process and the selectivity of such effects are still being studied.(Cahill & McGaugh, 1998; Benno Roozendaal, McEwen, & Chattarji, 2009; O T Wolf, 2009)

In the central nervous system glucocorticoids play an essential role in memory consolidation, for which emotional arousal is required also.(McGaugh, 2004; B Roozendaal, Griffith, Buranday, De Quervain, & McGaugh, 2003) Object recognition tasks, suitable for habituation, are able to focus on this role of arousal induced noradrenergic activity in memory enhancement.(Benno Roozendaal et al., 2006) The influence of glucocorticoids on enhancement of memory consolidation has been described by quite a few studies already. On the other hand, at the same time and involving the same brain regions and hormones, retrieval of emotional memory is impaired by increases of glucocorticoid hormones.(de Quervain et al., 2003; Benno Roozendaal & McGaugh, 2011) These findings have led to the hypothesis that acute stress modulates memory processes. Namely, it enhances consolidation, while retrieval suffers.(Benno Roozendaal & McGaugh, 2011) See figure 1. (de Quervain, Aerni, Schelling, & Roozendaal, 2009)

The endocannabinoid (eCB) system has been associated with anxiety and arousal during novel situations. The cannabinoid type 1 (CB1) receptor is also expressed highly in the amygdala, hippocampus and connected brain regions involved with memory processing.(Svíženská, Dubový, & Šulcová, 2008) In the central nervous system it predominantly consists of the endogenous cannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG) which activate the CB1 receptor. There, this receptor is predominantly found in regions involved with motivation, emotion and emotional learning.(Herkenham et al., 1990) The eCB system is also highly conserved among mammals, but not yet as well understood as the stress response. Recently however, it has been linked to glucocorticoid effects.(Matthew N Hill & McEwen, 2009) It would be really interesting to

know how the eCB system is involved with regulating memory processes. If more is known about this mechanism, we could discover a role in memory pathologies or whether it is a suitable target for therapeutic purposes.

Many effects of glucocorticoids on memory processes can be explained by genomic regulation. Consolidation takes time and improved performance on retention testing later on can be explained by genomic changes set in motion at acquisition. It should be considered that it takes time before gene expressions are changed and neuronal signalling is influenced by it accordingly to cause these effects. This does not correspond with retrieval impairments that already manifest at the time of risen glucocorticoid levels. Receptor mediated signalling cascades are a more likely cause.(Dallman, 2005; Tasker, Di, Malcher-Lopes, & Malcher Lopes, 2006) A model for central regulation of the stress response by the eCB system is described by Hill and Tasker and with these findings in mind possible mechanisms can be hypothesized.(M N Hill & Tasker, 2012) The effects of acute stress on retrieval of memories is such a direct effect, that regulation at the synapses is more obvious. The eCB system has qualities that make it suitable for fine-tuning neurotransmission. For instance, retrograde transmission of eCBs can regulate pre-synaptic neurotransmitter release. Emotional memory following acute stress could be influenced through synaptic transmission. Such a process would be very usable for pharmaceutical interventions, but we have to know how this works.(Fong & Heymsfield, 2009; Fride, 2005) A model for the role of the eCB system in memory consolidation caused by acute stress is depicted in figure 2.(Matthew N Hill & McEwen, 2009; Morena & Campolongo, 2014)

Research questions and hypotheses

The study in this report is part of the encompassing research looking into memory modulation by glucocorticoids. How are memory processes modulated by emotions? What physiology underlies this adaptability? Another topic it touches focuses on the memory process of retrieval. Whereas increased glucocorticoid levels increase memory consolidation, they have an opposite effect on retrieval. The former effects on memory can be attributed largely to transcriptional regulation. This study however, involves short term effects. Our hypothesis is that the eCB system, via in particular the cannabinoid type 1 (CB1) receptor, mediates such a fast effect. The rationale being that this is a G protein-coupled receptor that can activate receptor signalling cascades.(L de Oliveira Alvares, Pasqualini Genro, Diehl, Molina, & Quillfeldt, 2008) Another hypothesis addressed, is that different kinds of memory are not equally affected. Sensitivity is regulated at the synapses by an interplay of glucocorticoid, noradrenergic and eCB signalling. Also, the mentioned sensitivity should show memory effects with moderate dosages already and what is more, high dosages might even show adverse effects.(Park et al., 2006) Otherwise the results cannot be extrapolated to the natural and fundamental mechanisms under study.

To be more precise, this study focuses on object recognition memory and object location memory. The first research question is: does an increase of corticosterone in rats impair retrieval of object recognition and location memory within an hour? A second research question addresses whether blockade of the CB1 receptor reverses such an impairment. SR141716, also known as Rimonabant, is an inverse agonist for the CB1 receptor and used to block eCB signaling in this study.(Fong & Heymsfield, 2009, p. 948) Thirdly, is there a difference between the effect of corticosterone on object recognition and object location memory?

Our hypothesis is that corticosterone impairs memory retrieval of both object recognition and object location memory. Furthermore, the impairment is prevented by SR141716, which thus leads to memory performance similar to a control group. The impairments are limited to emotional memory and common sense dictates that not all retrieval is compromised, but then what kind of memory is not affected? Or are these types of memory affected to a different extent? Location and object recognition are both important aspects of experiences involving acute stress. They depend on different brain regions and have separate connections to the hippocampus. Read the appendix for a model of brain regions involved with recognition. The hypothesis is that memory retrieval performance is affected by corticosterone for each memory type, but the impairment itself or prevention of the impairment with SR141716 has a different impact. The latter could point to a mediating and decisive role for the eCB system rather than solely facilitative.

With the following experiments previously stated hypotheses are tested. Two different tasks for the subjects in the experiment required different types of recognition memory, namely for characteristics of objects or for the location of objects. When the outcomes of the experiments are compared, this difference reflects a difference in brain structures used. This report discusses the retention testing for rats to recognize objects with intervention of three dosages of corticosterone to assess the influence of glucocorticoids on memory performance. Consequently, with or without CB1 receptor blocker SR141716 intervention to assess involvement of the eCB system therein.

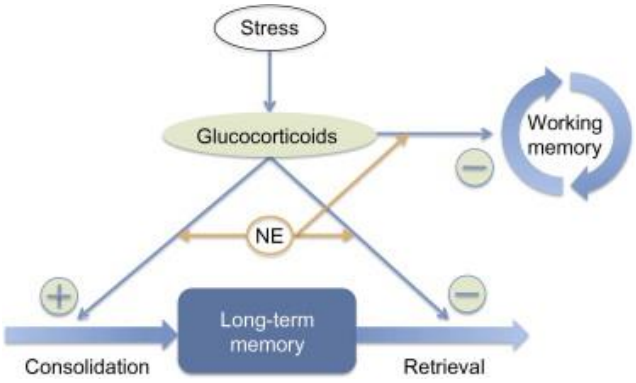

Figure 1. Model for the effects of acute stress on memory. Only when emotional arousal induces activation of noradrenergic transmission. NE, norepinephrine. Copied from De Quervain et al, (2009).

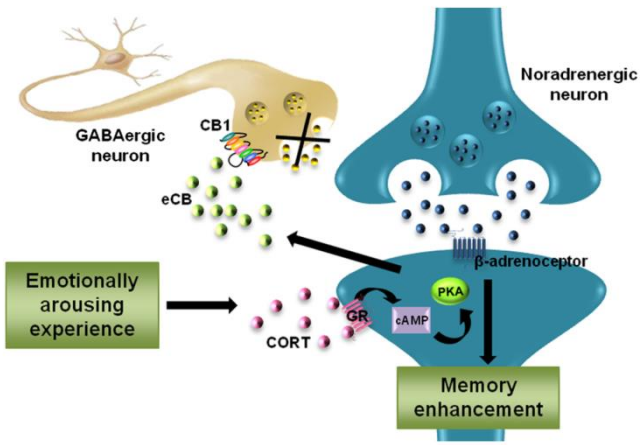

Figure 2. Model for the mediating role of the eCB system within the basolateral amygdala. Activation of glucocorticoid receptors results in eCB synthesis. ECBs bind CB1 receptors on GABAergic terminals, thereby inhibiting GABA release. Disinhibition of noradrenergic neurons increases norepinephrine release, enhancing the consolidation of emotionally aversive memories. Copied from Morena & Campolongo (2014) and adapted from Hill and McEwen (2009).

MATERIALS AND METHODS

Animals.

140 Male adult Sprague-Dawley rats (350-430 g at time of training), from Charles River Breeding Laboratories (Kisslegg, Germany) were kept individually in a temperature-controlled (22 °C) colony room and maintained on a standard 12 hr light : 12 hr dark cycle (07:00-19:00 lights on) with ad libitum access to food and water. Training and testing were performed during the light phase of the cycle between 10:00 and 16:00 h. All procedures were performed in compliance with the European Communities Council Directive (86/609/EEC) and were approved by the Institutional Animal Care and Use Committee of the University of Groningen, The Netherlands (DEC 5457 X).

Drug Treatment.

The cannabinoid receptor antagonist rimonabant (Kemprotec Limited, Middlesbrough, UK) or/and corticosterone (Sigma Aldrich) was dissolved in 5% polyethyleneglycol, 5% Tween-80, 5% ethanol and 85% saline and administered subcutaneously in a volume of 2.0 ml/kg, 60 minutes before the retention testing. The vehicle solution contained 5% polyethyleneglycol and 5% Tween-80, 5% ethanol in 85% saline.

Recognition Testing.

Behavioural tasks were conducted in a gray open-field box (40 cm × 40 cm × 40 cm). Bedding of the floor consisted of clean sawdust. The lighting of the room was diffuse. Two kinds of objects could be used as stimuli: either white glass light bulbs with a metal base (11 cm x 6 cm Ø) or transparent glass vials with a black plastic lid (5 cm x 5.5 cm Ø). For the training trial, the rat was placed in the experimental apparatus and able to explore two identical objects (A1 and A2) for 10 min. To avoid the presence of olfactory trails, sawdust was cleaned and stirred. The objects were thoroughly cleaned with 70% ethanol in between each trial. Subjects that showed no interest in the objects during training (6) or testing (11) would be excluded from the data. 20 rats were tested per session, always including some control animals and all dosages of corticosterone.

Retention was tested 24 hr later. For object recognition testing, one copy of the familiar object (A3) and a new object (B) were presented at the same two locations as the stimuli (A1 And A2) were located during the training trial. For object location retention testing one copy of the familiar object (A3) was placed in the same location while another copy of the familiar object (A4) was presented at a new location. All combinations and locations of objects were counterbalanced to reduce potential biases due to preference for particular locations or objects. In the retention test, the rat was placed in the experimental apparatus for 3 min. The time spent exploring each object ($t_{(NEW)}$ or $t_{(FAMILIAR)}$) and the total time ($t_{(TOTAL)}$) spent exploring both objects were recorded. Where the *NEW* points either to the new and different kind of object presented or to the object with a new and different location from the training stage. Exploration of an object was defined as pointing the nose to the object at a distance less than 1 cm and/or touching it with the nose. Solely turning around, climbing or sitting on an object was not considered exploration. A discrimination index could thusly be calculated via the calculation:

Discrimination index = $[t_{(NEW)} - t_{(FAMILIAR)}] / t_{(TOTAL)}$

For the object recognition groups this meant the difference in time exploring the novel versus familiar object. For the object location groups it is the difference in time exploring the object at the novel versus the familiar location. Indices are expressed as the ratio of the total time spent exploring both objects. A disadvantage of calculating retention with indices is that animals with little interest in the objects would dramatically influence the result. Therefore, rats showing a total exploration time of less than 10 s on training were excluded. Such eventualities have to be thought of beforehand as much as possible. When a researcher decides to exclude animals later on, it is much harder not to influence the results subjectively than when selection criteria are thought of beforehand. Behavioural experiments are even more prone to such errors since self-will cannot be predicted.

Statistics.

All data are expressed as the mean plus and minus the standard error of the mean. Data were analyzed by one- or two-way analysis of variance (ANOVA), followed by planned paired or unpaired student t tests when appropriate. One-sample t tests were used to determine whether the discrimination index was different from zero. A probability level of <0.05 was accepted as statistically significant.

In each experiment, training and retention test freezing behavior were analyzed using one way or two-way ANOVAs. Further analysis used Fisher's *post-hoc* tests to determine the source of the detected significances. In order to determine whether learning had occurred, paired *t*-tests were used to compare the training and retention latencies. For all comparisons, a probability level of <0.05 was accepted as statistically significant. The number of rats per group is indicated in the figure legends and ranged from nine to fourteen per group.

Data presentation

For the presentation of results bar graphs are chosen. In each of our experiments six differently treated groups are tested. Our hypothesis focuses on the role of Endocannabinoids. Therefore the measurements for groups in which we block the CB1 receptors are paired with the corresponding group that has unaltered eCB signalling, emphasizing potential differences. Bar graphs provide a suitable means to present the data, because it will be clear what group a measurement belongs to and measurements can easily be compared with any group. Most results do depend on specific experimental conditions, but relative differences should not. With this presentation our results are comparable with other experiments. The standard error of the mean fits in as well and incorporates different group sizes. Bars do not present the values of the data as precise as possible, but we are interested in relative differences, which are obvious in this manner. To compensate for this, the significance of notable differences is explained in the figure legend. The memory effect of GCs can also be distinguished easily by comparing bars of the same colour.

RESULTS

Most of the following results are a quantification of behaviour. Thus we define a certain kind of behaviour as described in our methods and measure the duration during a test. For measuring memory we make use of the novelty preference behaviour. Rats explore new objects longer and more intensely than previously encountered objects.(Ennaceur, Neave, & Aggleton, 1997, pp. 519–20) According to these statements, exploration time of the object is a valid method to measure memory. Other behaviours are all measured to take side effects into account. Examples are total exploration time, total active time, quadrant crossings, locomotion. Another measurement to control for unexpected effects is corticosterone level in the blood. The bioavailability of stress hormones is the sum effect of both administered CORT and endogenously synthesized glucocorticoids. Considering that too high concentrations often have adverse effects (Morena & Campolongo, 2014, p. 48; Park et al., 2006) and the result correlates directly with *Total* blood hormone levels, such a control is necessary to validate the effect of our intervention. This is also why two dosages of CORT (0.3 or 1.0) are chosen to be administered. It is not yet possible to predict which dosage is effective or simulates natural acute stress effects best. The higher dose might cause a stronger effect but can also weaken it. Especially since we are interested in the interplay between multiple neurotransmitter systems, the sensitivity for unwanted dose responses increases. Either way, testing with both dosages will lead to more interesting results and more information on the dosage effect on memory and behaviour.

Plasma corticosterone levels increase with administration of CORT

For the experiments in this report, rats were administered vehicle solution alone or with 0.3 or 1.0 mg/kg CORT before testing. These dosages were given either alone or in combination with SR141716 (1.0 mg/kg). Resulting plasma corticosterone levels of these treatments were measured from trunk blood as depicted in figure 3. Measurements are taken one hour after administration similar to the timing of retention testing.

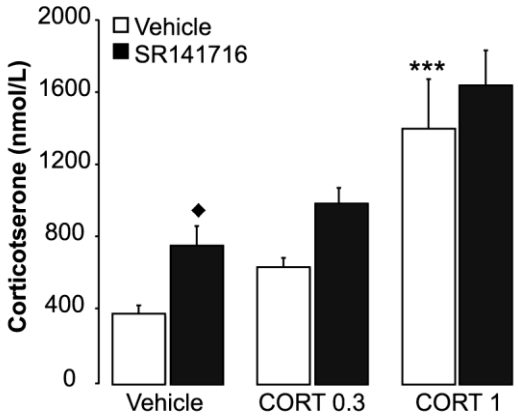

Figure 3. Plasma corticosterone levels of the rats one hour after administration:

- ☐ Open bars, vehicle alone or with CORT (0.3 or 1.0 mg/kg).
- ■ Filled bars, idem, but with SR141716 (1 mg/kg) as well.

♦, $p < 0.05$ compared to corresponding vehicle alone; ***, $p < 0.0001$ corticosterone groups compared to vehicle. All data is presented as mean ± SEM (n = 9 -15/group).

For the groups with only CORT (figure 3: open bars) the plasma corticosterone concentrations are dose-dependently higher. Although 0.3 mg/kg CORT did not significantly differ from vehicle alone ($P = 0.06$), it does show a higher mean. The 1 mg/kg CORT shows a greater increased effect on plasma corticosterone, with strong significance compared to the vehicle ($P < 0.0001$). The groups with also SR141716 administered with CORT (figure 3: filled bars) also show dose-dependently higher levels of plasma corticosterone. The dose-effect curve of the groups with SR141716 is similar, but less pronounced.

Notably, the group with only SR141716 shows an increased plasma corticosterone level versus vehicle alone ($P < 0.05$). The plasma corticosterone of the other groups with SR141716 show a trend of higher means on top of the dose dependent effect of CORT. The groups of 1 mg/kg CORT either with or without SR141716, were both significantly higher than the vehicle groups. The highest mean, the 1 mg/kg CORT group with SR141716, was not significantly different from the 1 mg/kg CORT alone group ($P = 0.24$).

The measurements of plasma corticosterone level of the groups with 1 mg/kg CORT administered have larger standard deviations. The standard deviations are similar in proportion to their corresponding absolute values as compared to the other measurements.

Object recognition memory: systemic SR141716 counteracts corticosterone-induced retrieval impairment.

Retention tests were carried out 24 hr after training. For the Object Recognition behavioural experiments rats were administered with vehicle alone, or with CORT, or also with SR141716 one hour before retention testing. SR141716 (1.0 mg/kg) blocks CB1 receptors. The corticosterone groups are assumed to exhibit retrieval impairments. If such results are shown, we can show by co-administration with SR141716 whether eCBs play a role in the mechanism. Substances were administered systemically via subcutaneous injection.

In training trials the exploration time of the identical objects was measured for each subject. During ten minutes of available time the rats spend 29.5 s (± 1.4) exploring the objects. Afterwards we could analyze these numbers between groups. With two-way ANOVA tests we found no differences between subjects that later on received vehicle or corticosterone and SR141716 (corticosterone: $F_{2,55}$ = 0.05, P = 0.94; SR141716: $F_{1,55}$ = 0.01, P = 0.93; corticosterone x SR141716: $F_{2,55}$ = 2.61, P = 0.08).

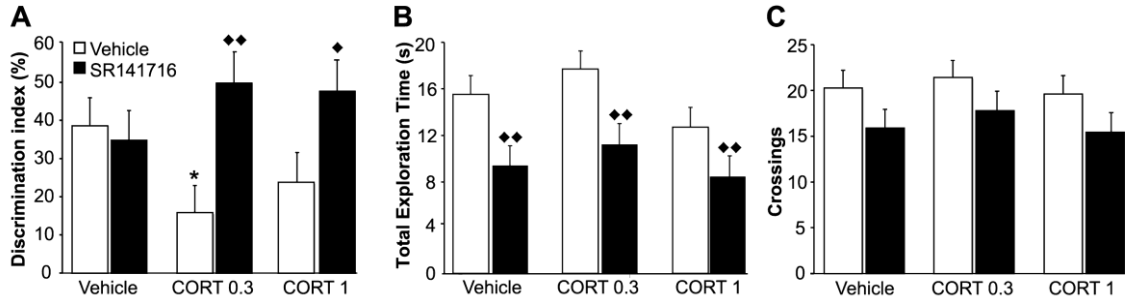

Figure 4: Retrieval of object recognition memory is impaired by corticosterone administration, but successful when co-administered with SR141716. (A) Discrimination index (%) as a measure for recognition memory. Groups were administered CORT (0.3 or 1.0 mg/kg) alone or together with SR141716 1 hr before retention test. (B) Total exploration time (s) of whichever object during retention test. (C) Number of quadrant crossings in the retention testing box during experiment.

♦, p < 0.05; ♦♦, p < 0.001 SR141716 groups compared to the corresponding CORT alone. *, p < 0.05 Corticosterone compared to vehicle alone. All data is presented as mean ± SEM (n = 9 -12/group).

Main measurement of the retention trials is the discrimination index. It represents the capacity to retrieve training memory. To give an example: a discrimination index of 50% shows that the attention time given to the new object is three times as long versus the familiar.

The groups of the object recognition experiment can be compared as shown in figure 4A. First of all, both groups without corticosterone have comparable discrimination indices. They indeed do not differ significantly from each other. These control groups significantly differ from zero (one-sample *t* test, P < 0.0001), which indicates successful discrimination of the objects. Groups with systemic corticosterone alone have lower indices. When looking at these CORT groups, open bars in figure 4A, 0.3 mg/kg CORT differed significantly from vehicle (P < 0.05), while 1.0 mg/kg CORT did not. Discrimination indices of around ~20% indicate that the CORT groups without SR141716 still have some successful retrieval of object recognition memory, but weaker compared to vehicle alone. Actually the 0.3 mg/kg CORT group did not significantly differ from zero (one-sample *t* test, $t_8 = 0.75$, P = 0.47).

The groups with SR141716 (figure 4A: filled bars) behave notably different with CORT. Discrimination indices of both 0.3 and 1.0 mg/kg CORT groups are relatively high. These values are not significantly greater than the groups without CORT, but do appear higher. They are significantly higher than the corresponding means of groups with CORT though. The means of CORT 0.3 groups differ from each other with $P < 0.001$, the CORT 1.0 group means differ from each other with $P < 0.05$.

One of the control measurements was the Total Exploration Time of the objects, of which for this test the results are shown in figure 4B. Some variation is seen between the different groups. When comparing the group with vehicle alone and groups with CORT alone, the CORT 1 group animals have shown the lowest exploration of the objects around 12s. No significant effect of corticosterone was found though (two-way ANOVA, $F_{2,55} = 2.61$, $P = 0.09$), the groups do not differ from each other. All the group means of SR141716 treated animals are lower than the vehicle or CORT alone groups. The difference between vehicle and SR141716 groups is quite significant, because $P < 0.0001$ (two-way ANOVA, $F_{1,55} = 16.71$). No interaction effect is found ($F_{2,55} = 0.24$, $P = 0.78$).

We can additionally see, looking at the absolute values of object exploration, that only a small amount of the total test time is spent exploring the objects specifically. Other behaviour was categorized as exploring the box, ground, walk around or rest. Crossing from one quadrant of the box to another area is a measure for activity and exploration of the experimental box. It also shows some variation, illustrated in figure 4C. One can observe a trend of lower means for animal groups treated with SR141716, because animals show fewer crossings. Two-way ANOVA demonstrates the drug effect for SR141716 is significant ($F_{1,55} = 6.11$, $P = 0.01$), but corticosterone did not show a drug effect ($F_{2,55} = 0.56$, $P = 0.57$). Once again no interaction effect is observed ($F_{2,55} = 0.01$, $P = 0.98$).

Object location memory: no corticosterone-induced retrieval impairment with systemic SR141716.

The object location memory experiment follows the same set-up as previous object recognition experiment. Retention tests were carried out 24 hr after training. Groups are administered with vehicle solution alone, corticosterone, with or without SR141716 via subcutaneous injection. If CB1 receptors mediate retrieval impairments caused by corticosterone, we hope these results will demonstrate. These results can then be compared with the object recognition study to shed light on differences between affected brain regions and memory components.

During the ten minutes of training and habituation average exploration time of the two identical objects was 33.7 s (±1.6). No effects of group allocation or treatments later on were found. Two-way ANOVA calculations established $F_{2,61}$ = 2.47 and P = 0.09 for corticosterone animals, $F_{1,61}$ = 0.12 and P = 0.72 for later SR141716 administration. No interaction effect either ($F_{2,61}$ = 1.89 and P = 0.16).

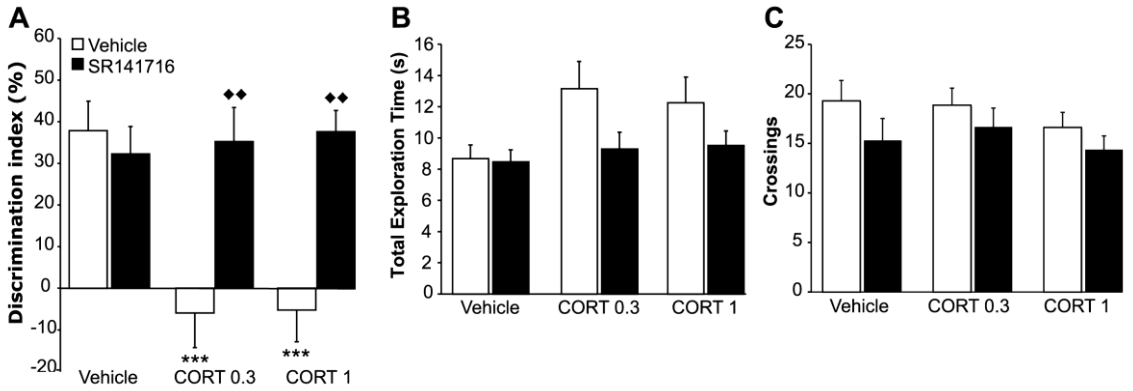

Figure 5: Retrieval of object location memory is impaired by corticosterone administration, but locations are discriminated by rats when co-administered with SR141716. (A) Discrimination index (%) as a measure for object location memory. Groups were administered CORT (0.3 or 1.0 mg/kg) alone or together with SR141716 1 hr before retention test. (B) Total exploration time (s) of whichever object during retention test. (C) Number of quadrant crossings in the retention testing box during experiment.
♦♦, $p < 0.001$ SR141716 compared to the corresponding CORT alone; ***, $p < 0.0001$ CORT groups compared to vehicle alone. All data is presented as mean ± SEM (n = 9 - 14/group).

The meaning and use of the discrimination index is explained before. Here it applies to identical objects that have different locations, one of which is new.

In figure 5A discrimination indices are compared after retention testing. Open bars represent groups with subsequently vehicle alone, 0.3 and 1.0 mg/kg CORT. In the vehicle alone group a discrimination is apparent. The mean differs from zero according to a one-sample t test which calculated t_9 = 4.90 with $P < 0.001$. The groups with CORT however did not discriminate. Actually, calculation with one sample t testing calculated no significant distinction from zero as t_{13} = -0.70 with P = 0.49 for the 0.3 mg/kg CORT group and t_{12} = -0.66 with P = 0.51 for the 1.0 mg/kg CORT group, thus no discrimination was measured for those. When we compare these three groups, it shows that the groups administered with corticosterone alone have impaired discrimination indices (compared to vehicle

alone $P < 0.0001$). These results are strengthened by statistical analysis with two-way ANOVA showing a significant main effect of corticosterone ($F_{2,61} = 4.20$, $P = 0.02$).

Co-administration with SR141716 shows a different picture. Represented by the filled bars in figure 5A, the discrimination indices of all three groups seem alike and similar to vehicle alone. Indeed none of them differs significantly from the control group ($P \geq 0.62$). For SR141716 treated rats we have measured successful discrimination, with or without heightened corticosterone levels. Two-way ANOVA analysis shows a significant main effect ($F_{1,61} = 17.47$, $P < 0.0001$) and furthermore a significant interaction between SR141617 and CORT with $F_{2,61} = 6.18$ and a $P = 0.004$. When SR141716 is compared with 0.3 and 1.0 mg/kg CORT the difference is clear and significant with $P < 0.001$ for both. In summary, the results in figure 5A show impaired retention test performances for corticosterone alone groups. Co-administration with SR141716 results in successful location memory retrieval comparable to control.

Exploration time of both objects is once again an important control measurement. Both objects should be explored for a little time to be able to discriminate them. The rats need to have had a significant time of interest in the objects for results. Other thoughts when looking at this control are whether activity, concentration, alertness and such factors that can influence our measurement besides memory are altered. A factor that can be influenced by corticosterone is fear and thus related behaviour.(Sandi & Pinelo-Nava, 2007; Oliver T Wolf, Atsak, de Quervain, Roozendaal, & Wingenfeld, 2015) Especially since rats exhibit open field avoidance behaviour, it is considered for this test in which location is of such importance. It is taken into account by selecting an experimental apparatus that is not too big and placing all objects within "sniffing distance" of the border. Total exploration time is somewhat less when compared with the recognition test results. Total exploration time was affected by SR141716 ($F_{1,61} = 5.26$, $P = 0.02$), but no effect of corticosterone has been found with two-way ANOVA ($F_{2,61} = 2.72$, $P = 0.08$). There was no interaction effect either ($F_{2,61} = 1.15$, $P = 0.32$).

The number of quadrant crossings during the retention test shows similar effects. SR141716 showed an effect ($F_{1,61} = 4.20$, $P = 0.04$) and these results are therefore shown as well. Corticosterone once again did not have an effect ($F_{2,61} = 1.07$, $P = 0.38$) nor did two-way ANOVA statistical analysis show an interaction effect ($F_{2,61} = 1.17$, $P = 0.83$). The reducing effect of SR141716 on quadrant crossings can be interpreted as a decreased exploration of the whole test area and apparatus. This result is a recurrence of measurements in previous object recognition test, indicating a common effect of SR141716, which is just one of the interesting results that we can take a closer look at in the following chapter of discussion.

DISCUSSION

Our results have shown us what effects the corticosterone administration has on plasma corticosterone levels. Since corticosterone is readily transported across the blood-brain barrier(Pardridge & Mietus, 1979), it can easily be imagined how the interventions of this study affect the glucocorticoid hormone level of the rat and its availability in the brain. Our results in addition with previous studies carried out with corticosterone make it possible to evaluate neurotransmitters that modulate memory processes. Moreover the role of SR141716 in memory retrieval and both object recognition memory and object location memory in particular. We believe that differences in the outcome of object recognition and object location experiments are caused by differences in the brain regions in question. Different kinds of information rely on brain regions in a different way and memories will even be stored via different types of connections, as depicted in the appendix. Does the brain alter which kind of memories it processes during acute stress? This study examines the role of endocannabinoids and can give way to new theories on how memories are processed and modulated.

It is not yet known how the brain treats different types of input or information exactly. Much has been found out about regional dissimilarities in the neuroanatomy that provide an advantage in processing the input. Via neurotransmitter systems that can reach multiple regions, situation specific "states" of the brain adapt to processing input in the most appropriate manner. While looking at behaviour, this study aims to focus on a smaller scale, namely two regions (or clusters thereof) that are involved with memory retrieval. One entails neurons that recognize object features, the other experiment tests for neurons that recognize the previous location of objects. As explained in detail in the introduction retrieval depends on other processes that facilitate sensory input, acquisition and consolidation. This is of importance in interpreting the results, because we can look at underlying processes by using neurotransmitters and blocking receptors for them. Precisely these transmissions and parameters supporting it, are ideal targets for fine-tuning memory processing. Altering receptors, sensitivity or neurotransmitter release can change what is stored and when and this in turn affects behaviour based on these memories. Involvement of more neurotransmitters and receptors means a greater possible variety in how the brain uses information.

Plasma corticosterone levels increase with CORT administration, but are also affected by SR141716.

Our assumption has been that we could mimic increased plasma corticosterone levels caused by acute stress. The tested hypothesis is: CORT 0.3 groups have higher corticosterone plasma levels, unaffected by SR141716. The graph results (figure 3) are interesting and the importance to have this control is clear when we see the difference between vehicle alone and SR141716. The significant effect of SR141716 and the difference between the groups without CORT indicates that SR141716 influences corticosterone, anxiety or sensitivity to environmental factors previously mentioned. According to the graph, the vehicle SR141716 group might compare better with the CORT 0.3 alone group with significantly similar corticosterone values. Anyway, SR141716 exhibits some side-effects and it is no surprise that it has effects on behaviour and brain processes by itself. It has mainly been investigated for its effect on energy metabolism and feeding behaviour.(Fong & Heymsfield, 2009) These could also be influenced via corticosterone changes, but in this study we like to see the effects on memory and just have to keep these issues in mind when interpreting the results. Other studies are looking into this by administration directly into the brain via a cannula.(Lucas de Oliveira Alvares et al., 2010) An indication to brain region specificity of memory effects is therefore of extra interest. Because the vehicle SR141716 group has a higher mean corticosterone level, the difference with high dosage CORT groups is smaller.

Of significant importance are the plasma level comparisons with the CORT 1.0 alone group. First of all, it is significantly higher than vehicle group, but not from the CORT 1.0 SR141716 group, in line with the hypothesis. This gives a good base and background for the upcoming results. When the corresponding values are not straight-forward, the lower dose groups can be looked at for additional information. The CORT 0.3 alone group does hold to the trend of dose responsiveness, but is not significantly different according to our statistical analysis.

In summary, the interventions have a clear effect on corticosterone levels. Either with or without SR141716 the corticosterone levels increase similarly with increased dosages of CORT. It is therefore justifiable to evaluate the effect of corticosterone in each series. For the effect of increased corticosterone with or without SR141716, it is best to first look at the high dose. The CORT 1.0 groups have both significantly different corticosterone blood levels from the vehicle groups without CORT.

The controls for activity, namely *Total exploration time* and *Number of crossings*, show an overall trend where SR141716 treated groups score lower results. Statistical analysis only verifies a difference for the *Total exploration time* of the Object recognition test, but the trend is seen for each group and its corresponding group with the same dosage of CORT. The exploration was rather high in the vehicle groups of the Object recognition experiment. In comparison with the groups in the Object location experiment this was high as well. Lower times on the other hand are more prone to variation. It depends on observed behaviour and the calculated index is less precise when a lower value is measured. No interaction effect was calculated for each series; therefore the effect is specific to SR141716. All in all, this does not indicate any influence on memory directly. Especially since each intervention has a control with a group that only gets vehicle solution instead of hormones dissolved in vehicle. It does however make interaction effects more important. Without interaction the effect cannot directly be assigned to memory process changes.

Object recognition memory: Endocannabinoids mediate the impairing effect of corticosterone. With SR141716 the ability to retrieve is restored.

The goal of this experiment was to induce impairment of recognition memory by administration of CORT. Besides this, we expected a recovery of retrieval performance when CB1 receptors were blocked. Our hypothesis states that modulation of recognition memory by glucocorticoids is mediated by eCBs and their receptors. Our results can give an idea of how strong this effect is and how eCB transmission is involved.

Our results show two significantly similar performing vehicle groups. If corticosterone levels are increased by SR141716, as previously discussed in our plasma control experiment, this increase is either not strong enough to impair retrieval or the effect of increased corticosterone levels is diminished by SR141716. The latter seems plausible to me in view of the following discussion. Exploration times measured are lower than for vehicle groups and therefore SR141716 is not inactive.

The impairment is successfully induced after CORT administration, as seen in figure 4A. We expected a stronger impairment with our hypothesis, but positive discrimination values show worse performance rather than complete impairment. Some recognition remains despite elevated corticosterone levels. Unfortunately the CORT 1.0 alone group does not even show a significant difference with the vehicle groups. The measured mean is lower, but not significantly so. An interim conclusion can be given, that a higher blood level of corticosterone does not strengthen the memory impairment for object recognition. Thus, either higher administrations of CORT were less effective in impairing the retrieval of memory in our animals or the impairment does not affect object recognition as much as other types of memory.

An experiment with more statistical power could discern if the CORT administered groups differ from each other as to their performance. It would be interesting to know what kinds of memories are used to still be able to discriminate and which skills are affected by the corticosterone impairment. Another factor could be the mean exploration time, which is somewhat low in this group. However, it does not seem to have affected the standard deviation more than other groups and the restrained result cannot be explained thusly.

When CB1 receptors are blocked by SR141716, the effect of corticosterone is almost reversed. Instead of impairment, rats with both CORT and SR141716 actually show better performance. Figure 4A shows both groups with CORT and SR141716 (filled bars) have similar performance and the highest discrimination in comparison with the rest. Although their means are not significantly different from vehicle alone or vehicle with SR141716, they are very much different from their corresponding groups that had CORT alone. As the mean discrimination indices are almost 50%, they gave three times as much explorative attention to the new object.

The effect of SR141716 on corticosterone memory impairment is not caused by a more passive attitude of the rats. Since the decreased total exploration time of SR141716 groups did not give rise to differences between the vehicle alone group versus the vehicle with SR141716 group. Because the graph also shows more significant differences in the groups treated with CORT 0.3, this supports our hypothesis that higher administration of CORT is not necessary. We did not see a great effect on

control measurements, but my view on these mechanisms is that they are more subtle and therefore higher dosages do not reflect the system we want to describe.

Considering the plasma results from before, we can also compare the vehicle SR141716 group with the CORT 0.3 alone group. These had similar corticosterone blood levels during the blood plasma level control measurement and here they show recognition memory impairment without SR141716 and retrieval performance similar to the vehicle group with SR141716. This would imply that a rise of corticosterone by itself causes recognition memory impairment, combined with SR141716 this impairment is blocked and with even higher levels of corticosterone the net result is actually better recognition memory.

Not to forget, the mechanism acts via controlling norepinephrine transmission. The decrease of exploration time is worrying, because it shows an imbalance in the system in a different area than we are focused on. There could be a role therein for norepinephrine, via corticosterone or an effect of eCB transmission by itself. But then again, when one looks at interaction effects, one can expect to see some systemic effects on other areas.

Object location memory: Retrieval is impaired by corticosterone via endocannabinoids and blocking the CB1 receptor lifts this.

Goal of this experiment was to alternatively focus on location memory and show the role of eCB transmission in its impairment by corticosterone. So, induce the impairment and measure location memory retrieval. We hypothesize decisive mediation of the response by the eCB system. Afterwards we can then compare with previous object recognition memory results. The experiments together can give a far better idea about local effects of the impairment. Differences can be attributed to the memory mechanism. Any side effects of the treatment would affect both experiments and is unlikely to influence one more than the other.

Control measurements are vital to check our assumptions. The hypothesis predicts a similar exploration time graph and idem for the number of crossings. First of all, the number of crossings in figure 5C shows a very similar picture indeed. A trend of less active rats when treated with SR141716 is again noticeable, but not significant. This can be attributed to metabolic effects of eCB transmission and this result should not influence memory performance. The total exploration time of the objects (figure 5B) however is somewhat lower than previous experiment. This might give rise to a different distribution since animals with too little interest were excluded. Also, the groups without CORT do not show a difference in exploration. Of course a new object might be more interesting than a familiar object at a new place. The difference is not statistically significant and therefore no underlying causes can be appointed yet.

The groups without CORT administration show similar performance between them, figure 5A. The new object is readily discriminated. If the vehicle with SR141716 group caused higher corticosterone levels by the latter, this does not result in a different average performance. CORT administration causes complete impairment of discrimination. Both groups with CORT alone are not significantly different from zero, whereas the corresponding vehicles are. The different dosages do not influence performance. Both groups with CORT have the same performance without SR141716 and also the

same performance with SR141716. This can mean that corticosterone is modulatory active by small changes in concentration and the effect does not reverse with our higher dose.

Regardless of treatment influences on corticosterone blood levels, SR141716 has a strong effect on corticosterone induced location recognition impairment. SR141716 blocks the impairing effect of corticosterone completely and consistently. Consolidation takes place between training and test. No intervention has taken place during this time; therefore this must be a retrieval specific effect. Faulty consolidation could otherwise give performance issues as well. Altogether, without CB1 receptor transmission, corticosterone cannot impair retrieval of location memory.

Discrimination between object recognition and object location memory retrieval during acute stress.

Between both retention test tasks the results were quite dissimilar. The overall performance of control groups is slightly better for object recognition. It cannot be concluded that rats have better memory for objects than location, because it depends on our measurement method. Another method for retention testing might yield different results, our choice is perhaps just better suited for object recognition. The problem is that many other factors have influence. This has also resulted in a difference in control measurements. Especially the total exploration time is slightly better for the object recognition experiment. No difference in the performance of the groups without CORT for the object location experiment is actually the odd man out. Surely, we hoped to see no effect at all on total exploration time of SR141716, but the object recognition experiment demonstrated otherwise.

How do these experiments connect with the way we use our memory? The types of memory this experiment researched are typically essential in emotional experiences. Object recognition is a strong stimulus for emotional memory retrieval. It depends on the perirhinal cortex for example and other cortical components involved with semantic memory. These handle a big part of the memory aspects that encompass the 'what'. Locations are also strong triggers for remembering an experience. This memory component relies more on the hippocampus and parahippocampal gyrus, which engages with the 'where'. Although there are a lot of differences between the rat brain and the human brain, the basic mechanisms are similar. So maybe the components affected in rats relate to slightly different components in the human brain, but they can be modulated in the same way. Especially since both the corticosteroid and the eCB system are well conserved in mammals. The network that handles object location memory retrieval was completely deficient during the emotional aroused state and also the mechanism facilitating object recognition retrieval was impaired. Thus, during acute stress the brain might focus on other memory processes, but retrieval takes a temporary loss.

The eCB system has been shown to be directly involved with regulating memory retrieval performance during acute stress. Previous studies underscore the presence of CB1 receptors throughout the brain with dense concentrations in the areas of interest for these experiments. Since it is shown that the CB1 receptor mediates the effect of corticosterone retrieval impairment, it might as well contribute to which brain regions are sensitive for corticosterone modulation. After all, without eCB transmission this modulation is not possible. Its role in mediating the effect of corticosterone also makes it an interesting target for therapeutic interventions. Our results suggest

that location memory retrieval is more sensitive than object recognition memory retrieval. What is more, if the same is true for consolidation effects, these processes are most likely connected.

Technical considerations

Hormone levels change under influence of all kinds of factors. First of all, there is a daily rhythm that can vary blood concentration levels tremendously. With females in particular many changes are due to longer cycles, individual differences therein and environmental factors. Secondly, environmental factors that include diet, climate and visitations or attention from humans. Most of these factors can be normalised in the laboratory environment. The time of testing could influence corticosterone levels, even though tests are performed during day time (when rats rest) for this reason. Lastly and most importantly, since corticosterone is very dependent on anxiety this can very well mean that animals already have heightened hormone levels during testing. Especially since animals are handled more intensively during this period. Prior to training, habituating interaction aims to decrease anxiety induced by handling, while maintaining the necessary excitement for norepinephrine release. Ideally the subjects are given exactly enough corticosterone to reach similar blood levels for each group. However, this would lead to variations in the given treatment, require invasive measurements during testing and burden the animals increasingly. Subjects might also still have varying degrees of sensitivity for the hormone, which would even be difficult to take into account. Thus, a control test measuring corticosterone blood levels afterwards and having a vehicle group with a normal variation of endogenous corticosterone levels is a good approach.

If open field avoidance would have been an issue, we could expect no impairment effect in case the object placed at the new location was more accessible, no discrimination in case it was vice versa, or large differences between individual rats. No such behaviour or result has been found and it is safe to state that this did not bother the animals. Crossing quadrants during exploration of the experimental apparatus and no lingering support this. The box was no open meadow to them.

There are still more factors that could play a role in the results or for instance the measured exploration times. Videos of the training and tests are reviewed by the researcher. In many cases this was done by myself and checked or repeated by my supervisor. Some differences were observed between measurements. This means that there is a subjective component to these experiments. Also, I noticed that there is a learning curve both in carrying out the experiments as in judging the behaviour of animals. This was considered and researchers have been trained with assessing old footage and compare the scores with the scores of experienced researchers till matching results were achieved. Still, many experiments were carried out and assessed at different times, which can cause small deviations. I do not think that this changes the results, but I do consider that each single result is in fact a collection of significant figures. Namely, many consecutive time measurements make up the whole for each animal. This is not immediately apparent when one looks at the figures and should be kept in mind.

IN CONCLUSION

It has astonished me that behavioural experiments can be used to answer such profound questions. Via pharmaceutical intervention and our knowledge of neurotransmitters and receptors we can selectively alter the biological system. It is valuable to be able to test in a fully functional living organism. This makes the results translatable to the complex interactions we have with our environment and elucidates how changes can affect our behaviour, revealing many effects that might otherwise pass unnoticed. By selecting the right behavioural experiment for the job a researcher can further narrow down what brain functions will be focussed on.

Of utmost importance is that a lot of control measurements are needed to discriminate the result of interest from side effects and systemic interference. Amusingly, discriminating objects was in fact the exact task we asked of our rats. The downside to this may be apparent. A cognitive task measured via observation of behaviour can be influenced by many issues besides memory function. Without a proper background one cannot start such experiments, since this study also relies heavily on previous work. Experience is essential as well to anticipate on environmental factors. All in all, I think this study was done quite successful, for all approaches were considered with much thoughtfulness.

The approach of the study has borne fruit, since it produced quite a few significant differences between the result of intervened test groups and the behaviour of control animals. First of all, the increasing effect of SR141716 on corticosterone blood levels was surprising. It almost led me to believe the given CORT concentrations should have been adjusted downward for these groups. However, this would not have been possible for the group that did not receive any CORT. It has changed the way I reviewed the behaviour test results in a big way though. Fortunately, any performance that contradicted effects of risen corticosterone can be taken extra seriously. Also we measured with two CORT concentrations given, so one can compare with a higher dosage in many cases.

Why SR141716 changes blood corticosterone levels in rats is not clear from this study. Speculating on this, the cause may lie in the direction of metabolic changes. Both substances are highly interactive with metabolic processes and these properties of SR141716 could influence in turn corticosterone levels. In another line of thought, SR141716 has a way to influence corticosterone availability in the central nervous system. eCBs have shown to be highly interactive with corticosterone signalling and the norarenergic system. This is the disadvantage of systemic administration, whereas local administration would narrow down the possibilities and maybe even eliminate the effect in case the former explanation was true. Studying the time course profile of corticosterone increase after SR141716 administration could be another approach. Since the tasks in this study require so many cognitive abilities to work together, it is better we started with the whole brain under influence of corticosteroids like during acute stress. Now we studied the effects without missing connections to regions we do not know to be involved yet. A more selective focus on regions indicated in this study would definitely be an interesting next step however.

The hippocampal formation is with its surrounding brain areas optimally connected to modulate behaviour and learning processes. Object recognition processing follows a dorsal path for spatial information, and a ventral pathway that relays object characteristics. For consolidation and memory these pathways run from the visual cortex via the inferior temporal cortex to the medial temporal lobe. This study focuses on the latter region where the amygdala also lies and mediates emotional

responses with memory. It is fascinating how the brain unravels sensory information and combines the input with memory information to guide our behaviour and consciousness. Therefore it has been very interesting to see different results between the experiments with object changes and location changes.

The corticosterone influences both object recognition and location recognition processes. Increased levels impair location retrieval more dramatically. A different dosage effect does not seem to be the case, since higher dosage does not impair object recognition more. Perhaps some pathways that process object recognition are not susceptible to corticosterone or overall sensitivity to the effect is less. In general object appearances are better memorized than their location, since locations change more often. This study shows that during circumstances of arousal and acute stress one or more brain areas involved with retrieval of object location are completely impaired or add up to it. Especially the hippocampal area has the receptors for glucocorticoids, but it is not yet clear which neurons and connections determine this susceptibility to impairment.

The increased performance of the groups with both CORT and SR141716 suggests that the eCB system inhibits optimal performance. This is not implausible since its proposed mechanism in consolidation involves inhibition via GABA neurotransmission. Strangely the control group with only SR141716 did not show an increase in performance suggesting no decrease of GABA inhibition during low levels of corticosterone.

Also blockade of the CB1 receptor has a few side effects, such as reduced total exploration time. In the object recognition test this is already seen with the administration of SR141716 alone. It did not change performance with the vehicle alone group however. In my opinion, these side-effects did thusly not concern the memory measurements.

It has been tough to discern changes to our area of interest from the bigger behavioural picture. There is no clear boundary between memory, behaviour and systemic effects. The experiments led to a good understanding though of how each experience is processed differently. The eCB system definitely plays a role in the processing of emotional memories. It is of most use to compare these results with neuroanatomical knowledge. It would be interesting to see what happens when in every case, from consolidation to retrieval, the impairments would be lifted. Is it possible to increase both retrieval and consolidation at the same time? Or are these impairments connected in such a way, that one cannot have it both ways. Or, finally, would this result in the uncontrollable recurrence of emotional experiences not unlike post-traumatic stress disorder pathology.

Since discrimination indices of both 0.3 and 1.0 mg/kg CORT groups are significantly higher than their corresponding vehicles with CORT, this study shows that the CB1 receptors and eCBs mediate the corticosterone modulation effect. It makes these components interesting targets for therapy and is a nice addition to the workings of recognition memory. The human brain has a lot of capacity available for recognizing objects, make associations and whole areas are dedicated to facial recognition even. Recognizing our sensory inputs is thus an integral part of our consciousness. It is nice we have successful tools to learn more about this.

AFTERWORD

For my master's degree programme in Biomedical Sciences I wanted to focus on the brain. Topics related to cognition, memory and how to modify brain function interest me in particular. How can a brain structure operate and where do these processes depend upon? First I found an internship at the department of medical genetics, examining protein aggregation in neurons. For the second part I have shifted my attention to brain anatomy and behaviour. The group of Benno Roozendaal at the Universitair Medisch Centrum Groningen did just that, with fundamental research about the mechanism of memory processes and more specifically in relation to stress. I also wanted to get a better understanding of how behavioural experiments can lead to a better understanding of underlying brain processes. The practical work involved with behavioural experiments and examination of the rat brain appealed to me as well.

For this study I have been taught and supervised by dr. Piray Atsak. Her PhD research focused on the eCB system when I joined her work for my internship. I have had previous experience in research on the effects of recreational drugs on brain function. Firstly thus, I associated the endocannabinoid system with the effects of cannabis, also known as marihuana. Indeed it is known that this neurotransmitter system affects mood, pain, appetite and memory. The latter piqued my interest, because I want to know what mechanisms facilitate memory processes. And if endogenous eCBs and their corresponding receptors can influence memory formation or extinction, what is the biological background to these interactions and how can it affect our experiences? Finally, one of my favourite themes is the possibility of enhancing memory or supporting it by targeting such a system.

REFERENCES

Cahill, L., & McGaugh, J. L. (1998). Mechanisms of emotional arousal and lasting declarative memory. *Trends in Neurosciences*, *21*(7), 294–299.

Dallman, M. (2005). Fast glucocorticoid actions on brain: back to the future. *Frontiers in Neuroendocrinology*, *26*(3–4), 103–108.

de Oliveira Alvares, L., Engelke, D. S., Diehl, F., Scheffer-Teixeira, R., Haubrich, J., de Freitas Cassini, L., … Quillfeldt, J. A. (2010). Stress response recruits the hippocampal endocannabinoid system for the modulation of fear memory. *Learning & Memory (Cold Spring Harbor, N.Y.)*, *17*(4), 202–209.

de Oliveira Alvares, L., Pasqualini Genro, B., Diehl, F., Molina, V. A., & Quillfeldt, J. A. (2008). Opposite action of hippocampal CB1 receptors in memory reconsolidation and extinction. *Neuroscience*, *154*(4), 1648–1655.

de Quervain, D. J., Aerni, A., Schelling, G., & Roozendaal, B. (2009). Glucocorticoids and the regulation of memory in health and disease. *Frontiers in Neuroendocrinology*, *30*(3), 358–370.

de Quervain, D. J., Henke, K., Aerni, A., Treyer, V., McGaugh, J. L., Berthold, T., … Hock, C. (2003). Glucocorticoid-induced impairment of declarative memory retrieval is associated with reduced blood flow in the medial temporal lobe. *The European Journal of Neuroscience*, *17*(6), 1296–1302.

Ennaceur, A., Neave, N., & Aggleton, J. P. (1997). Spontaneous object recognition and object location memory in rats: the effects of lesions in the cingulate cortices, the medial prefrontal cortex, the cingulum bundle and the fornix. *Experimental Brain Research*, *113*(3), 509–519.

Fong, T. M., & Heymsfield, S. B. (2009). Cannabinoid-1 receptor inverse agonists: current understanding of mechanism of action and unanswered questions. *International Journal of Obesity (2005)*, *33*(9), 947–55.

Fride, E. (2005). Endocannabinoids in the central nervous system: from neuronal networks to behavior. *Current Drug targets.CNS and Neurological Disorders*, *4*(6), 633–642.

Herkenham, M., Lynn, A. B., Little, M. D., Johnson, M. R., Melvin, L. S., de Costa, B. R., & Rice, K. C. (1990). Cannabinoid receptor localization in brain. *Proceedings of the National Academy of Sciences of the United States of America*, *87*(5), 1932–1936.

Hill, M. N., & McEwen, B. S. (2009). Endocannabinoids: The silent partner of glucocorticoids in the synapse. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(12), 4579–4580.

Hill, M. N., & Tasker, J. G. (2012). Endocannabinoid signaling, glucocorticoid-mediated negative feedback, and regulation of the hypothalamic-pituitary-adrenal axis. *Neuroscience*, *204*, 5–16.

McGaugh, J. L. (2004). The amygdala modulates the consolidation of memories of emotionally arousing experiences. *Annual Review of Neuroscience*, *27*, 1–28.

Morena, M., & Campolongo, P. (2014). The endocannabinoid system: An emotional buffer in the modulation of memory function. *Neurobiology of Learning and Memory*.

Nathan, S. V, Griffith, Q. K., McReynolds, J. R., Hahn, E. L., & Roozendaal, B. (2004). Basolateral

amygdala interacts with other brain regions in regulating glucocorticoid effects on different memory functions. *Annals of the New York Academy of Sciences*, *1032*, 179–182.

Pardridge, W. M., & Mietus, L. J. (1979). Transport of steroid hormones through the rat blood-brain barrier. Primary role of albumin-bound hormone. *The Journal of Clinical Investigation*, *64*(1), 145–54.

Park, C. R., Campbell, A. M., Woodson, J. C., Smith, T. P., Fleshner, M., & Diamond, D. M. (2006). Permissive influence of stress in the expression of a U-shaped relationship between serum corticosterone levels and spatial memory errors in rats. *Dose-Response : A Publication of International Hormesis Society*, *4*(1), 55–74.

Roozendaal, B. (2002). Stress and memory: opposing effects of glucocorticoids on memory consolidation and memory retrieval. *Neurobiology of Learning and Memory*, *78*(3), 578–595.

Roozendaal, B., Griffith, Q. K., Buranday, J., De Quervain, D. J., & McGaugh, J. L. (2003). The hippocampus mediates glucocorticoid-induced impairment of spatial memory retrieval: dependence on the basolateral amygdala. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(3), 1328–1333.

Roozendaal, B., McEwen, B. S., & Chattarji, S. (2009). Stress, memory and the amygdala. *Nature Reviews. Neuroscience*, *10*(6), 423–33.

Roozendaal, B., & McGaugh, J. L. (2011). Memory modulation. *Behavioral Neuroscience*, *125*(6), 797–824.

Roozendaal, B., Okuda, S., Van der Zee, E. A., & McGaugh, J. L. (2006). Glucocorticoid enhancement of memory requires arousal-induced noradrenergic activation in the basolateral amygdala. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(17), 6741–6.

Sandi, C., & Pinelo-Nava, M. T. (2007). Stress and memory: behavioral effects and neurobiological mechanisms. *Neural Plasticity*, *2007*, 78970.

Svíženská, I., Dubový, P., & Šulcová, A. (2008). Cannabinoid receptors 1 and 2 (CB1 and CB2), their distribution, ligands and functional involvement in nervous system structures — A short review. *Pharmacology Biochemistry and Behavior*, *90*(4), 501–511.

Tasker, J. G., Di, S., Malcher-Lopes, R., & Malcher Lopes, R. (2006). Minireview: rapid glucocorticoid signaling via membrane-associated receptors. *Endocrinology*, *147*(12), 5549–5556.

Westlake, T. M., Howlett, A. C., Bonner, T. I., Matsuda, L. A., & Herkenham, M. (1994). Cannabinoid receptor binding and messenger RNA expression in human brain: An in vitro receptor autoradiography and in situ hybridization histochemistry study of normal aged and Alzheimer's brains. *Neuroscience*, *63*(3), 637–652.

Wolf, O. T. (2009). Stress and memory in humans: twelve years of progress? *Brain Research*, *1293*, 142–154.

Wolf, O. T., Atsak, P., de Quervain, D. J., Roozendaal, B., & Wingenfeld, K. (2015). Stress and memory: A selective review on recent developments in the understanding of stress hormone effects on memory and their clinical relevance. *Journal of Neuroendocrinology*.

APPENDIX

While writing this report I became especially interested in the fundamentals of the endocannabinoid system and the neuroanatomy of recognition memory. This appendix contains some of the pictures I found during my research that gave me a deeper understanding into the brain matter.

Hippocampal endocannabinoid receptor distribution

Cannabinoid receptors are common in the brain as seen in figure i below.

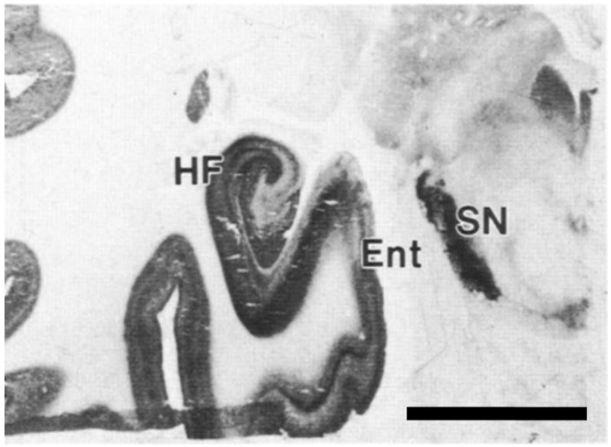

Figure i. Distribution of CB receptors via autoradiography with cannabinoid [‘H]CP-55,940. Coronal plane section cut of human brain. Midline is to the right. Ent, entorhinal cortex; HF, hippocampal formation; SN, substantia nigra; Scale bar = 10 mm.

From Westlake et al. figure 1c (Westlake, Howlett, Bonner, Matsuda, & Herkenham, 1994).

Object recognition

Object recognition neuronal information flow. The order in which visual sensory input is processed towards recognition memory is simplified as follows:

From the eye retina neural signals are send via the *thalamus*, to the V1 *visual cortex*. Through processing by deeper visual cortical layers V2 - V4, axons guide signals to the *inferior temporal cortex.* This region processes complex visual information before it is send to memory processing areas. The neural path continues to the *medial temporal lobe* with the *hippocampus* and surrounding memory processing regions, like those in figure iii.

I found the following figure ii a nice illustration of how brain regions are connected. This shows efferent projections from the hippocampal formation, central in the neural network for memory processes.

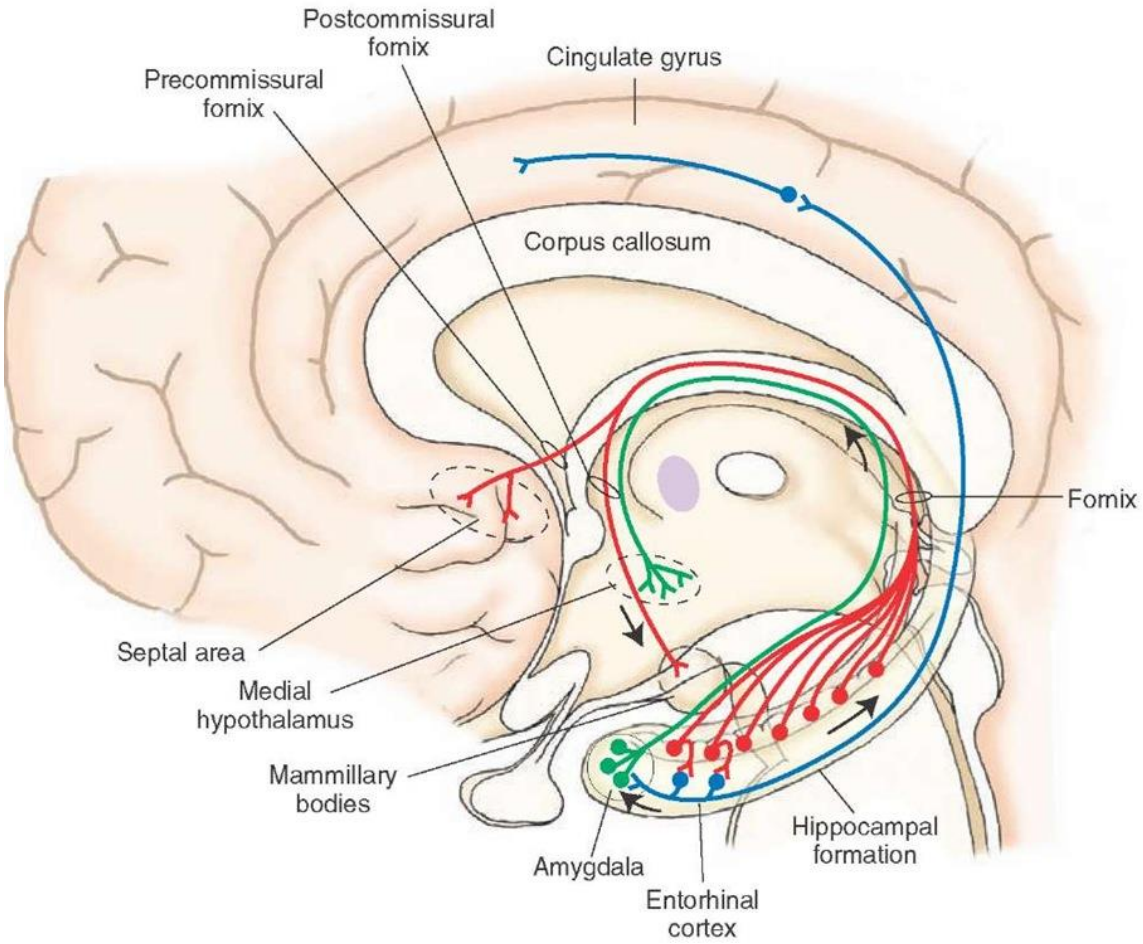

Figure ii. Major projection targets of the hippocampal formation. The primary output is through the fornix to the diencephalon (i.e., medial hypothalamus, mammillary bodies, and anterior thalamic nucleus) via the postcommissural fornix and to the septal area via the precommissural fornix. Other connections shown include efferent fibers that synapse in entorhinal cortex, which, in turn, project to amygdala and cingulate gyrus.

http://what-when-how.com/neuroscience/the-limbic-system-integrative-systems-part-1/

From the Atlas of the Human Brain I looked up where the *entorhinal cortex* (eRC), *perirhinal cortex* (pRC) and *parahippocampal gyrus* (pHG) are situated in the brain and how they are connected. This gives an idea of how these abstract models are related to real brain anatomy.

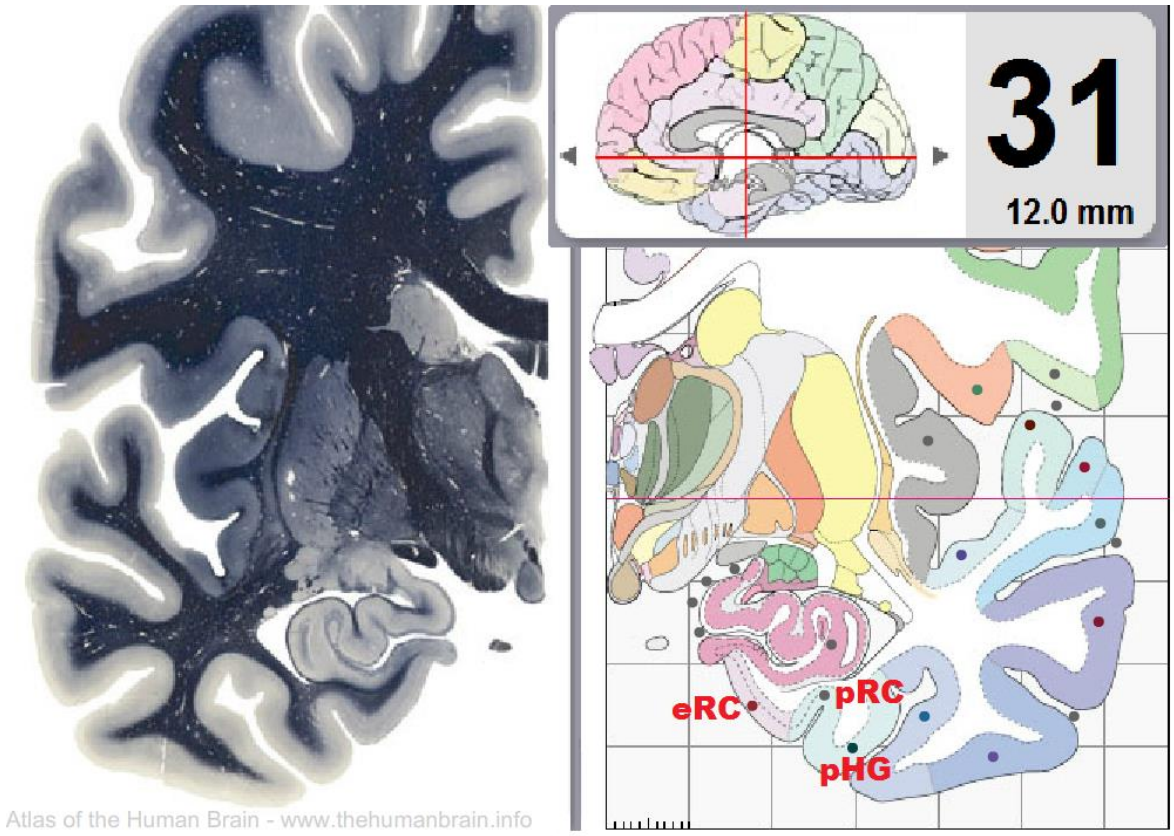

Figure iii. Object recognition memory regions surrounding the hippocampus. Adapted from the online atlas at www.thehumanbrain.info.