Bylage 3.

REGISTRATION FORM ANIMAL TESTING

(version September 2010)

PROTOCOL NUMBER:	RECEIVED 1st Version: 20 mei 2010
DED-201	2nd Version:

1. **Algemene gegevens.**

1.1 **Title.** Effects of stress hormones on risky decision-making and memory consolidation

1.2 **Onderzoekinstituut** (indien van toepassing):

Thema: Animal physiology and Cognitive Neuroscience (APCN)

1.3 **Indien het onderzoek het maken van genetisch gemodificeerde muizen betreft, vermeld dan het nummer van de vergunning van het Ministerie van Landbouw, Natuurbeheer en Visserij (BBD-nummer).**
Not applicable.

1.4 **Indien het onderzoek genetisch gemodificeerde muizen betreft, vermeld dan het nummer van de vergunning van Commissie Genetisch Gemodificeerde organismen (COGEM) (GGO-nummer).** Not applicable
Wat is de inschaling van de COGEM?

2. **Uitvoerende onderzoekers**

2.1 **Naam** :
Opleiding :
Tel. Nr :

Kamernummer:
e-mail:

2.2 **Organisatorische werkeenheid.**
Naam afdeling :
Adres :
Kostenplaatsnummer :

2.3 **Overige medewerkers.**

Naam	Tel	Opleiding	Art 9/Art 12/Geen
		Biology Neuroscience HLO Neuroscience & Pharmacology (UMC Utrecht) Neuroscience	Art 9 Art. 9 Art 12 Art 9 Art 9

* Paraaf van verantwoordelijke artikel 12 functionaris op handtekeningenblad

3. Gegevens over het doel van de dierproeven.

3.1 Uiteindelijk doel (objective of the research approach).

The overall goal of this project is to examine the effects of stress hormones on risky decision-making and memory consolidation in rats, and to asses how these hormones affect the neural ensemble mechanisms underlying these two cognitive processes.

Corticosterone is the main glucocorticoid hormone in rodents and it is involved in mediating stress responses. Plasma corticosterone levels are elevated after stress. After passing the blood–brain barrier, the hormone binds to mineralocorticoid (MR) and glucocorticoid receptors (GR). Acute effects include increase of release probability of glutamate-containing vesicles, facilitation of long term potentiation and increased excitability in CA1 area of the hippocampus. In parallel with these rapid changes, corticosterone starts slower, gene-mediated processes. Long term exposure to stress induces structural changes and LTP impairment in hippocampus (Joels 2008), and structural changes in associative corticostriatal circuits and sensorimotor areas (Dias-Ferreira et al. 2009).

At a behavioral level, acute or chronic stress can both facilitate or impair performance depending on the timing of the stressor and the behavioral task and on the brain circuit activated by the behavioral task and the brain area where the stress hormones exert their action (Joels et al. 2006).

Several recent studies in our lab describe the involvement of orbitofrontal cortex (OFC) in signaling certain versus uncertain rewards (van Duuren et al. 2009), reward magnitude (van Duuren et al. 2008), and the implications of hippocampal replay during slow wave sleep for the association of spatial location with reward (Lansink et al. 2009).

Previous articles have shown alteration of behavior, with decision-making biased toward habitual strategies, following chronic stress (Dias-Ferreira et al. 2009), impaired memory retrieval (de Quervain et al. 1998) and audiogenic stress impairing hippocampal LTP and spatial memory (Kim et al. 2007). It is not known, however, what the effects are of corticosteroid receptor activation on risky decision-making and the underlying neural coding mechanism, and on memory consolidation and the associated process of ensemble replay in the hippocampus and OFC.

3.2 Direkt doel van de hieronder aangemelde dierproeven.

The goal of the project is to: (1) Develop a behavioral paradigm to identify the time-point of drug application with maximal effect on decision making and/or memory, and 2) conduct tetrode-array recordings in the OFC and hippocampus while rats perform the behavioral task.

Part 1) builds on existing literature. It has been shown that natural stress induces behavioral changes associated with alteration of network morphology in brain areas associated with decision making in rats (Dias-Ferreira et al. 2009; Liston et al. 2006). Also, local or systemic application of corticosteroids alters their feeding behavior (Sakai et al. 2000). Kim et al. (2007) show that audiogenic stress impairs LTP in slices and retention of spatial memory.

In the first phase of the project, in a series of behavioral tasks we will study how the stress hormone corticosterone (acting on GRs and MRs), injected at different time intervals relative to the onset of the behavioral task, will affect performance in a risky decision making task as well as memory for this task experience. We propose, therefore, to assess the timing of GR/MR signaling pathway activation and the consequences for behavior.

In the 2nd step the optimal parameters will be applied to rats trained on the same behavioral task. These animals will be subjected to tetrode-array recordings in two brain structures deemed important for decision-making and memory: the orbitofrontal cortex and hippocampus. The goal of the 2nd step is to record ensemble activity in OFC and hippocampus (i.e., spikes from multiple, discriminated neurons) during decision making under stress induced by corticosteroid injection, and relate the results to previous findings (Kim et al. 2007; van Duuren et al. 2009). These recordings will be used to study outcome-predictive ensemble coding in the orbitofrontal cortex during decision-making and for analyzing the interaction between stress and hippocampal replay in the awake and sleeping state after the task experience (Lansink et al. 2009). However, the possibility of replay occurring in the orbitofrontal cortex will also be studied, as well as the impact of risky decision-making on the hippocampus. Moreover, cross-correlations between the two brain structures will be analyzed for both cognitive processes.

We opt for rats as the animal model of choice because they have been frequently used to assess the impact of acute or chronic stress on behavior and reliable comparisons can be made with previous studies. Also, the tetrode recording technique is well developed in rats. It is important to study ensemble activity because a) the yield is much higher than in standard electrophysiological

recording techniques (up to 100 units per session with tetrode recordings compared to 1-3 units per session with standard single-electrode recordings), and b) it allows to dissociate the neural coding of value, or other decision variables on single-cell vs. network level (Roesch et al. 2006; van Duuren et al. 2007; van Duuren et al. 2008). The principal applicant has substantial experience in conducting tetrode recordings in rats and mice. Moreover, there exists year-long experience and profound expertise in the lab of Cyriel Pennartz to conduct this kind of research.

3.3 Vraagstelling(en) van het hier aangemelde onderzoek met event. deelvragen.
(1) How do corticosteroid receptors affect risky decision-making, and its underlying neural coding mechanisms in the orbitofrontal cortex?
(2) Which aspects of risky decision-making are coded in hippocampal representations?
(3) How do corticosteroid receptors affect memory consolidation and the associated process of ensemble replay in hippocampus and orbitofrontal cortex?
(4) Can memory consolidation be enhanced concomitantly with a stronger replay?

3.4 Is dit een pilot-experiment, nieuw onderzoek of een onderdeel van lopend onderzoek?
The pilot phase of the experiment will yield the optimal parameters for pharmacological manipulation, to be used in the second, ensemble-recording part of the study. Depending on the behavioral results, one of the two cognitive processes may be emphasized for further study (i.e. replay-consolidation or risky decision-making).
The second phase will involve tetrode recordings from OFC and hippocampus of pretrained rats. Stress hormone will be injected at only one preselected time point and the electrophysiological data will be recorded while animals perform the task. The necessary techniques build on methods well established in this research group (see e.g. protocols DED 119,109,110, Prof. Pennartz). No major modifications of the existing methods and technical protocols are expected.

3.5 Is hier sprake van (contract) research op verzoek van de industrie?

No. The project is sponsored by NWO (VENI-grant to TK & VICI-grant to CP).

Zo ja , is er een publicatiebeleid afgesproken met de sponsor?
(nb.: het recht om te publiceren mag niet blijvend worden uitgesloten) .
No.

Bent u vrij om de onderzoeksresultaten openbaar te maken? Yes

3.6 Welke resultaten of overwegingen hebben geleid tot het ontwerpen van deze experimenten?

The OFC has been repeatedly implicated in the representation of risky decision-making both in humans (Bechara et al. 1997; Bechara et al. 1996; Ernst et al. 2004; Rogers et al. 1999) and rats (Mobini et al. 2002; Pais-Vieira et al. 2007). These studies suggest that OFC is involved in estimating the reward value on the basis of its probability. More recent work (Kepecs et al. 2008; van Duuren et al. 2009) shows that neurons in the OFC are involved in signaling reward probability. Chronic stress alters decision making strategies (Dias-Ferreira et al. 2009) and impairs spatial memory by destabilizing firing rates of hippocampal neurons (Kim et al. 2007). The goal of this study is to find out whether corticosteroids affect risky decision-making and memory consolidation, and if these changes are visible by altered ensemble firing patterns in the OFC and hippocampus, both in awake and sleep states.

4 Beschrijving van het experiment.

4.1 Proefopzet (korte samenvatting).
It is important to describe the experimental design so that it is clear how the design will be able to answer the questions and which roles are played by the various experimental groups. In each **case** mention:
* The structure of the experiment
* Experimental conditions (independent variables) **per test group**
* Testable parameters (dependent variables) **per test group**
* The number of test animals required **per test group**

4.1.1 Behavior

Apparatus.
Rats will be trained and tested in the 'steering wheel maze' (SWM), which consists of a hexagonal runway giving access to 6 separate reward sites (A, B, C, D, E, F). Each reward site consists of a fluid-delivery well equipped with infrared photobeam detection for nose pokes. Upon nose-poking for a defined time period, the rat may obtain either a rewarding fluid (sucrose solution) or not (the volume of the solution applied can be regulated). Each reward site can be associated with a different type of outcome; e.g. site 1 and 2 may have 50% reward probability and 150 ul reward amount, whereas sites 3-6 have different values. During training, rats will be allowed to explore all of these various outcome options and are expected to develop preference for a particular site (longer time spent at the reward port, or shorter approach latency – time difference between cue light and arrival at the reward port).

Pilots.
The objective of the behavioral pilot phase is to find out how stress hormone (corticosterone) affects decision making and memory, and what are time points of injection, relative to the behavioral task, with the most pronounced effect. During the training phase, rats will be food-deprived until they reach 85% of their ad-libitum weight to motivate them to perform the task. Food deprivation will be adjusted daily to optimize behavioral output and at the same time maintain a high physical health status. For example, twelve hours prior to the start of the training total food restriction will be required (50% food restriction for the 24 hours prior to the start of the training). Subsequently the weight of the animals will be monitored daily and food adjusted until 85% of the ad-libitum wheight is reached. In a well-trained animal 10-15 grams of food daily normally provides a full caloric supplement to sucrose obtained on the task while maintaining good task performance. Thus, the performance during training as well as the subjects' weight, and any visible sign of health problems will be monitored daily and used to adjust food deprivation levels. For the behavioral pilot phase (ten groups of eight rats each), the training phase will be approximately 4 weeks per batch but, depending on how well the animals learn the task, could be extended up to 8 weeks. The behavioral testing will be approximately 2 weeks in order to obtain an assessment of the behavioral performance following injections at different time points.
We will also test the 'diet board' as food restriction tool. The diet board is thereby newly introduced to our lab. Because it has not been tested extensively, its impact on the motivation, stress levels and performance of the rats during the behavioral task are somewhat uncertain. Also, it requires a specific type of food pellets that are not readily available and, in our experience, difficult to obtain.
Given these aspects, we are going to test the diet board in a separate group of 5 rats in order to confirm its compatibility with our experimental approach. If the results are positive in this group and the logistic problems are solved we will implement the diet board for all animals.

Training protocol

Initial handling of animals.
Rats will arrive from the breeding labs at a weight of ca. 350g. In order to minimize the basal corticosterone levels during behavioral testing rats will be kept in a normal day/night rhythm (lights on at 7:00 Am). Food restriction required for a good task performance increases corticosterone levels at the beginning at the dark phase but does not influence significantly corticosterone levels an the beginning of the light phase (Stamp et al. 2008). Also the behavioral test involves two sleep sessions (immediately before and after the Steering Wheel Maze task) to study memory consolidation/replay during slow wave sleep. The sleep sessions need to be precisely timed with respect to the SWM task and they are easier to achieve in the rat's subjective resting phase (light phase).

Rats will be left to habituate to the housing conditions for one week. During this period they will be regularly handled and moved in order to get them used to the experimenter.

Pre-training
Rats will be subjected to a standard autoshaping procedure in which they learn the association between nose poke and fluid reward (10% sucrose solution). Naive rats will be placed into the SWM for 20 minutes each day. The cue lights of all nosepoke holes will be activated. Every coincidental nosepoke will be rewarded, i.e., cue lights will be switched off, the cue light at the reward site will be switched on, and 120µl sucrose solution will be available for a maximum of 20s. After reward

consumption or after 20s, the remaining reward will be flushed away by a vacuum system, and nosepoke holes will be activated again following an intertrial interval of 20-40s. If necessary, the task will be complemented by manual autoshaping, in which drops of sucrose solution will be put into the nosepoke hole to motivate the animals to make nose pokes. Moreover, the animals will be trained to maintain their nose in the nose-poke port for at least a minimum amount of time before they will receive the sucrose reward.

Choice behavior task.

After pre-training and autoshaping, different reward amounts will be introduced. Nosepoke holes will be associated with different reward amounts and reward probabilities (2 holes – 50 µl at high probability 75%; if rats prefer this, the characteristic is "safe", risk-avoiding choice behavior, 2 holes - 150 µl at low probability 25%, if rats prefer this, the characteristic is risky choice behavior, and 2 holes – 50 µl at low probability 25%, if rats prefer this unattractive option, the characteristic is habitual or indifferent behavior, insensitivity to reward value.). In a normal session, rats will run about 20 full laps (120 reward site visits possible). To prevent undersampling problems, we choose 3 reward conditions across the 6 sites (ie. each reward condition is represented by 2 sites, to get double sampling of each reward condition per lap). The sequence of combinations will be randomized within the animals across sessions. To avoid habit formation, we will quickly move on to the actual behavioral experiment (see next paragraph) once the rats show a small, but consistent preference for one of the reward conditions.

Corticosterone (CST) will be applied at three different time points relative to task onset: -3 and -0.5 hrs relative to task onset, plus the time point directly after the task. The -3 hrs-group is included to allow nongenomic effects of CST to fade out. Three Sham-injected groups will be included (injections at same time points), one naive group with no injections at all and three control groups where corticosterone, MR antagonist NR3C2 and GR antagonist RU486 will be injected at same three time points (ten groups of rats in total, about eight rats per group = 80 animals).

Corticosterone will be injected i.p. at 1 mg/kg (CORT in HBC-complex, Sigma cat. nr C-174). Following injection the free corticosterone levels in the hippocampus are somewhat higher than those measured after a 15 min force swim test and they return to baseline within one hour after injection (Droste et al. 2008).

At the end of the training sessions and 24 hours before the start of behavioral experiments session, blood will be sampled by tail incision to assess basal corticosterone levels – Cort_Baseline1 (if corticosterone levels turn out unexpectedly high, a pharmacological strategy based on GR or MR antagonists may have to be chosen). The procedure will be repeated (on alternate sides of the tail) one week later, directly after behavioral task, to determine corticosterone levels during experiments – Cort_Task, and 24 hours after the last behavioral experiment – Cort_Baseline2 - to asses a possible shift in baseline over the entire period of the behavioral testing (all animals will be tested between 8:00 AM and 12:00 PM – lights on at 7:00 AM) (see the scheme on page 16).

On each trial, animals make nosepokes to obtain one out of three possible sucrose rewards that differ in amount and probability. Table 1 lists the reward amounts and probabilities used, but parameters may be adjusted and refined during the pilot sessions. An important variable is the delay between nose poke onset in the well and time of fluid delivery. The choice of delay-times, reward probabilities and reward amounts is partly based on the literature, partly on experience in our own lab. Pilot studies in our lab have shown that rats can discriminate between rewards if the difference between the reward amounts is approx. 40µl or larger, also a delay of 2-3 seconds (but constant for all sessions) is optimal for evoking Go or NoGo choices from the central runway into a fluid-well exit. Furthermore, published results (van Duuren et al. 2009) show that animals respond differentially when presented with a 50%, 75% and 100% reward probability.

A typical experimental session can be summarized as follows. Rats will be subjected to a sequence of (1) pre-task rest/sleep (~1 hour), (2) task performance (~1 hour), (3) post-task rest/sleep (~1 hour) and (4) a memory retention test on the next day (free-choice trials to recall the most preferred reward site).

Table 1. Reward amount and delays used in the task

	Reward Amount	Probability
Nosepoke Hole A, B	50 µl	75 %
Nosepoke Hole C, D	150 µl	25 %
Nosepoke Hole E, F	50 µl	25 %

4.1.2 Electrophysiological experiments

The tetrode recording techniques that will be used in the task are standard techniques that are well established in our group (see e.g. protocols DED 119,109,110, Prof. Pennartz). However, even though the main applicant has extensive experience with these and other electrophysiological procedures in mice and rats, pilot studies with rats will be necessary to minimise drop-out during the actual experimental phase.

The main behavioural task will be identical to the choice behavior task described above. Sixteen rats (pretrained on the maze) will be implanted for dual-area recordings from orbitofrontal cortex and hippocampus. They will be subjected to the same session structure as above, except that injection of stress hormone (or sham injection, in counterbalanced sessions) will be done at only one preselected time point. In addition to the usual sessions with free-choice behavior, forced-choice sessions may have to be included in case rats tend to systematically skip certain reward sites. Replay processes will be studied (Lansink et al. 2009) in conjunction with assessment of consolidation, as tested behaviorally the next day in a retention probe test (again, equal reward conditions across all sites).

The training and testing phase will typically take about 4 weeks for each batch of rats. Subjects that do not attain a performance criterion (i.e., when their choice distributions are on chance level after 4 weeks of training), as well as those showing abnormal behavior (excessive grooming, excessive freezing, hyperactivity, etc.) will be excluded from further studies and will be made available for other experiments.

In order to publish a behavioral experiment, we need to include a minimum of 6 rats per group. Taking into account outliers and rats to be excluded for reasons mentioned above, we ask for a total of 16 rats for the electrophysiological phase. We ask for a maximum total of 101 rats; for the electrophysiological (16 rats), behavioral experiments (80 rats) and testing of the diet board (5 rats). We emphasize that we not plan to use all of these rats, but the outcomes of the various tests may necessitate going up to this maximum.

Rats will be implanted with electrodes for extracellular recording in the OFC and hippocampus (see work protocol at the end of this document for surgical procedures). The implanted device ("hyper-drive") contains 12 independently movable tetrodes (a special 4-channel electrode allowing the recording and discrimination of up to ~15 cells each) plus two reference electrodes.. Ad-libitum food will be available 2-3 days prior to surgery. After surgery, rats will be allowed to recover for 2-5 days, and will be administered analgesic medications. No experimental manipulations will take place during this period, except for the assessment of the electrophysiological signal quality and the gradual lowering of the electrodes towards the target brain structures. After implantation, animals will be housed individually to prevent damage to the implant. After the recovery period animals will be trained to support the weight of the tetrode drive during the behavioral task.

Once rats are fully recovered, and good electrophysiological signals are observed (typically two weeks after surgery), animals will begin food restriction, and the experimental manipulations will start. Corticosterone levels will be measured as described in the behavioral pilot phase.

Each experimental session will start in the morning, with the optimization of the electrode placement. The behavioral session with electrophysiological recordings, according to the protocol specified below, will take place a few hours later, in order to allow the electrodes to stabilize.

After animals have completed an electrophysiological experiment, electrode positions will be marked by passing a small amount of current through each tetrode. After a period of at least one day, the animals will then be sacrificed with a fatal dose of anesthesia, perfused with fixative, and the brain removed for histological slicing and staining.

5.1 Omschrijving te gebruiken diersoort(en) per experiment en/of per experimenteergroep.

	Groep Pilot (Behaviour) Experiment 1	Groep Electrophysiology Experiment 2
Diersoort	Rat	Rat
Aantal (MAXIMAL)	80	16
Stam	Wistar	Wistar
Geslacht	Man	Man
Leeftijdsrange	4-7 maanden	4-7 maanden
Gewichtsrange	300-375 gr	300-375 gr (at surgery)
Microb.status(*)	SPF	SPF
Herkomst	Harlan	Harlan
Lokatie		
Huisvesting (**)	Type 4 & 40x40x40 cm	Type 4 & 40x40x40 cm
Barrière (***)	Conventioneel	Conventioneel

(*) bij aanschaf: conventioneel/SPF/CRF/GB/GF
(**) kooitype (2/3/4/metabole) of afmetingen per kooi, evt afwijkende maat
(***) D1/D2/quarantaine, conventioneel, dag/nacht ritme, controle, handelingen, verzorging, etc.

5.2 **Periode waarin de dierproeven zullen worden uitgevoerd** (maximaal 4 jaar).
Startdatum: 01-07-2010
Afronding: 01.05.2013
(N.B. De aanmelding moet binnen 1 jaar na goedkeuring starten, anders moet het protocol opnieuw worden ingediend bij de wetenschapscommissie (ODP-leider) en de DEC.)

5.3 Plaats van uitvoering van het experiment.
(Indien radioactieve stoffen worden gebruikt, de aanmelding indienen bij de Algemeen Stralingsdeskundige (F1-112)).

6 Schatting van het ongerief.

6.1 Te verwachten risico van ongerief.
(noem alle aspecten per experimentele handeling en ook ongerief ten gevolge van de handeling)

For pilot behavioral experiment :
Shaping: Little discomfort (approx. 2-4 weeks)
Food-restriction: Little discomfort (approx. 8 weeks)

Exp. Handelingen	Groep Nummer	Kwalificatie ongerief Gering Gering tot matig Matig Matig tot ernstig Ernstig	Duur
Shaping, handling, pretraining	All animals (max. 101)	Little discomfort	Approx. 2-4 weeks
Behavioural training	All animals (max. 101)	Little discomfort	Approx. 2-8 weeks
Surgery	All animals in the electrophysiological study (max. 16)	Moderate discomfort, anesthesia injection (intra-peritoneal) head incision, craniotomy. micro-drive implant (performed under general anesthesia)	about 3 hours
Post-operational stage	All animals in the electrophysiological study (max. 16)	Moderate discomfort (first 24-48 hours; analgesic medications administered) to little discomfort	approx. 4-10 days
Experimental phase (i.e. rat is performing behavioral task with implanted tetrodes in cranium)	All animals in the electrophysiological study (max. 16)	Little to moderate discomfort	approx. 4-12 weeks
Solitary housing	Electrophysiology group (max. 16 or 96 depending on the food restriction method)	Moderate discomfort (animals can see, hear and smell each other)	approx. 5-13 weeks (including post-op stage)
Food-restriction	All animals (max. 101)	Moderate discomfort (Diet board or 0 -50% food restriction to maintain behavior, food available on tasks)	approx. 8-14 weeks
Total	(max. 101)	moderate to severe discomfort	Up to 17 weeks

6.2 Hoe lang zit het dier in een proef, gerekend vanaf de eerste handeling/ingreep aan het dier?
Up to 17 weeks for electrophysiology studies, up to 8 weeks for pilot studies

6.3 Indien in het kader van deze aanmelding genetisch gemodificeerde dieren worden gefokt: welke afwijkingen of ongerief samenhangend met de genetische modificatie zijn er te verwachten? In welk stadium treden die op? Hoe ernstig schat u het daarmee samenhangende ongerief in? N/A

7. Alternatieven/Beargumenteren van de proefopzet.

Volgens artikel 10, lid 1 van de Wet op de Dierproeven is het verboden een dierproef te verrichten voor een doel dat, naar de algemeen kenbare, onder deskundigen heersende opvatting, ook anders dan door middel van een dierproef kan worden bereikt.

7.1 Zijn er alternatieven voor deze dierproef(ven)?
Vervanging? At the moment, there are no alternative techniques capable of providing data about the relationship between behavior, learning, and neural code. Non-invasive techniques such as

neuroimaging, EEG etc. do not access the level of the information encoding at the level of detail necessary to evaluate the combined effects of learning and molecular manipulations. It is to be noted that the high data-yield of multi-tetrode recording techniques allows a significant reduction in the number of animals needed to attain statistical significance in neurophysiological measures.

Vermindering? Group sizes and the exact behavioral, neurophysiological measures necessary to study the relevant questions here will have to be evaluated. 6 - 8 rats is a typical group size for ensemble neurophysiology and behavior experiments. A precise study of statistical power will require the availability of the first electrophysiological data, but it seems likely that 8 rats per behavioral configuration represent a conservative maximum, and that fewer animals will likely be needed.
The aim of the project is to investigate specifically the effects of glucocorticoid and mineralocorticoid activation on decision making and memory consolidation. The direct delivery of the corticosterone is therefore preferred over a natural stressor paradigm that would produce a general stress response, which would involve additional physiological pathways (e.g. autonomic nervous system activation). Furthermore, it is not apparent from existing literature what is the precise temporal relationship between GRs/MRs activation and the effects on the cognitive processes described above.
Ten groups are therefore required for behavioral testing:

- three will be injected with corticosterone at different time points to estimate the injection timepoint with maximal behavioral effects;
- three groups will be injected (in parallel with the groups above) with corticosterone and GRs&MRs antagonists (at the same timepoints) to confirm the activation of the GR/MR pathways
- three sham-injected groups (at the same timepoints)
- one naive group (no injection)

The naive group (no injection) is necessary to assess the behavior in animals free of any stress and compare to the rest of the groups. It is necessary to estimate a baseline both for corticosterone levels and behavior during our specific experimental conditions.
The number of animals requested in the current protocol for the electrophysiological phase is slightly higher than in previous studies (e.g. DED 174) because it involves the use of a split-hyperdrive which targets two brain areas (hippocampus and orbitofrontal cortex) as opposed to the standard hyperdrive. Due to a more complex design, the use of a split-hyperdrive adds difficulty to the implantation and recording procedure. The uncertainty involved in using state-of-the-art *in vivo* electrophysiological methods justifies a maximum of 16 animals for the electrophysiological experiment. Furthermore, the added uncertainty of actual data yields means that the actual number of animals needed can only be determined by data analysis going on at the same time as the experiments.

Verfijning?
In order to validate and optimize the experimental design we will first begin by carrying out behavioral pilot experiments.

7.2 Indien van toepassing: hoe kunnen de resultaten van deze studie/dit onderzoek worden geëxtrapoleerd naar de humane situatie?

Yes, in large part. In humans stress alters behavior (Porcelli and Delgado 2009) showing poor performance on memory tasks and risk taking biases exaggerated under stressful conditions. Similarly (de Quervain et al. 1998) in rats memory retrieval and spatial memory (McEwen 2007) are impaired by glucocorticoids. Also, and decision making is biased towards habitual behavior after chronic stress (Dias-Ferreira et al. 2009).
Effects of glucocorticoids in humans on selective attention have been described as similar to changes in sensory integration and response selection in stressed animals (Lupien and McEwen 1997).
Although several studies have described the effects of stress on decision making, little is known about the effect of corticosteroids on coding of reward probabilities in OFC cells and hippocampal replay of behavioral sequences.
For obvious ethical and economic reasons, studies with monkeys are not a good substitute for experiments with rats, and the tetrode technology proposed here is better established for rats than for monkeys. Moreover, the neuroscientific methods used in human studies are, with very few exceptions, limited to non-invasive techniques. However, the dissection of choice behavior at the level of neural networks or populations with proper behavioral tasks and with fine spatiotemporal resolution (~ms) cannot be achieved with fMRI, PET, EEG or other methods suitable for use on

human subjects. Our invasive techniques are not possible on humans, except for exceptional cases, so that the extrapolation from animal studies is a necessary component of the study of these mechanisms in mammals in general, including humans. Furthermore, neurophysiological and lesion studies in rats, human, and other mammalian species have revealed basic similarities in the functioning of prefrontal cortex and limbic areas.

7.3 Kunnen dieren (door anderen) worden hergebruikt?
Naive animals used for pilot experiments (without injection and surgical implants) can be re-used in other studies. Implanted animals might be used to provide pilot data for other protocols, once they finish their experimental cycle, if necessary. However, in general it is essential to secure the brain tissue of implanted animals for histological controls.

7.4 Beargumenteer de keuze van de gebruikte diersoort(en).
The rat is the species of choice for behavioral electrophysiology, for its cognitive ability and flexibility, the ease of training, the physical resistance, and for the enormous deal of knowledge available about its anatomy and physiology. Previous studies have confirmed that the current protocols should be highly feasible in rats (van Duuren et al. 2008; van Duuren et al. 2009).

7.5 Geef een statistische argumentatie van de gekozen proefopzet en van de gekozen grootte van de experimentele groepen.

Choice behavior and memory consolidation tasks are well established in our lab, and more recent behavioral and electrophysiological studies suggest the involvement of rat OFC in decision making (Lansink et al. 2009; Roitman and Roitman; Seo and Lee; van Duuren et al. 2008) and effects of stress on decision making and memory (de Quervain et al. 1998; Dias-Ferreira et al. 2009; Joels et al. 2006; Sadowski et al. 2009).
Taking into account outliers (e.g., rats showing rigid, inflexible behavior or habits) a minimum of 8 rats per group (10 groups) is required for the behavioral pilot phase (total 80 rats for the behavioral pilot), Exp.1.
Based on previous experience in our lab, an average of 6 animals was used during tetrode recordings in orbitofrontal cortex in behavioral tasks similar to the one proposed here (van Duuren et al., 2007a, 2007b, 2008). Considering possible outliers during the behavioral testing of the implanted rats, 8 rats would be necessary for the electrophysiological phase (Exp.2). Due to a further drop-out rate of approx. 50% for technical or other reasons (see below), we estimate that a group of at least 16 rats will be necessary for the electrophysiological sessions (the use of a split hyperdrive, which targets two brain areas, adds to the difficulty of the surgery & recording - compared for example with DED 174). Each rat should yield 30-100 cell recordings per session over 6 -12 experimental sessions, for a total of 180-1200 cells (i.e. independent spike trains) per rat. About 500-5000 cells in total for each experiment offer a number that should allow good statistical power for the complex neural ensemble measures that we intend to compute. Because of the complex statistical analysis performed, a detailed power analysis is neither possible nor customary in this type of experimental work without any data available.
Because it is difficult to give an exact estimate of animals needed for both the behavioral and the neurophysiological part of the experiment, the figures presented here may be inaccurate. Additional animals may be needed in case of higher standard deviations or smaller effect sizes than expected.

7.6 Geef per experimenteergroep een schatting van het aantal proefdieren dat uitvalt (i.v.m. voortijdig overlijden, mislukken van het exp, etc) **en beargumenteer deze schatting.**

No losses are anticipated for the pilot behavioral studies (Exp.1), and the training phase of the electrophysiological tasks. Some animals will be dropped at the pre-training stage (and possibly re-used for other experiments) if they fail to meet the performance criteria and/or show signs of abnormal behavior. A small number of losses (about 1 in 10 - 20 subjects) are possible during surgeries (Exp.2). Some animals (around 2 in 10) may not yield usable electrophysiological data, because of factors such as small blood clots, scar tissue build-up etc., impairing the normal mobility of the electrodes and their electrical properties. The likelihood of this occurring is somewhat reduced by the refinement of techniques through ongoing studies in the lab and personal experience of the primary applicant. Finally, the longevity of the hyper-drive is variable, which may necessitate a larger group of animals. Overall, a conservative estimate of the drop-out rate is approx. 50% of the 16 animals requested for the electrophysiology experiments (Exp. 2).

8 Ethische afweging.

8.1 Wetenschappelijk belang van de hier aangemelde dierproeven.

Recent studies have shown that single cells and ensembles are coding for expected reward probability in the OFC (van Duuren et al. 2009). Also hippocampal replay during slow wave sleep has been suggested as a mechanism for memory consolidation (Ji and Wilson 2007) and the correct association spatial location to reward is essential for survival (Lansink et al. 2009).
Behavioral studies showed alterations in decision making and memory function (de Quervain et al. 1998; Dias-Ferreira et al. 2009; Porcelli and Delgado 2009) and a limited number of electrophysiological studies described changes in firing patterns of place cells in hippocampus under stress (Kim et al. 2007)
The current experiment sets out to investigate the role of single and ensembles OFC neurons during risky decision making under stress and how the coding pattern changes in relation to basal stress levels. Firing patterns of individual cells in the hippocampus, underlying memory formation and consolidation, will be studied in parallel before, during and after the behavioral task.
This experiment will therefore shed light on the functional changes of OFC and hippocampus during risky decision making and memory consolidation, respectively. Also it will help finding therapeutic targets for treating the cognitive consequences of stress.

8.2 Maatschappelijk belang van de hier aangemelde dierproeven.

Decision making under stressful conditions in some form pervades nearly every moment in daily life with somewhat higher incidence for certain professional categories. Good decisions, taken under stressful situations, are crucial for emergency-service personnel, stock-market brokers, air traffic controllers, etc.
These groups are required to handle large amounts of information, sometimes incomplete, in a relatively short time interval. Decisions have to be taken and updated, by comparing previous and current information, under acute or prolonged stress with significant impact in the prevention of injury and death, material damage or control of financial costs.
Furthermore, exposure to stress for prolonged time can lead to a series of chronic conditions (chronic head ache; anxiety disorder; memory disturbances; increased blood pressure, sugar and cholesterol; exacerbation of allergies; sleeplessness) with significant impact on the economy and quality of life.
Even though the outcomes of the current study will not directly contribute to finding new approaches in therapy, it will have significant implications for the clinical work. If our findings can indeed show whether and how the OFC and the Hippocampus subserve decision making and memory consolidation under stress, therapies can better take into account the specific characteristics of the OFC and Hippocampus and their target structures (e.g. striatum).

8.3 Geef aan waarom het belang van de voorgestelde proeven het gebruik van dieren en de mate van ongerief voor u aanvaardbaar maakt.

Currently, these invasive experimental techniques have no alternatives capable of providing the same kind of data on the neural code for reward and decision making, and how those directly relate to behavior. The mechanistic knowledge obtained with this kind of experiments is needed to lay the foundations for therapeutic (pharmacological/behavioral) advances. If successful, our results may suggest electrical or pharmaceutical treatments for disorders characterized by bad decision making, such as drug addiction/substance abuse, problem gambling, frontal lobe syndrome and mood and attention disorders. To minimize the discomfort following the admittedly invasive nature of the experiments, a number of steps will be taken. Analgesic and anti-bacterial prophylaxis therapies will be administered in the post-surgical stage. It has to be added that the high data yield of our techniques will allow the number of subjects needed to be reduced to a minimum, in comparison with those required for older methods.

9. **U wordt verzocht op dit blad (graag een aparte bladzijde) in een voor ieder begrijpelijke Nederlandse samenvatting van uw voorgenomen onderzoek te geven. Deze samenvatting van ten hoogste 200 woorden dient in ieder geval informatie te geven over:**
   - vraagstelling en methodiek,
   - direct en/of indirect nut voor de geneeskunde,
   - soort en verwacht aantal te gebruiken dieren,
   - ongerief van het dier

In dit onderzoek zullen we bestuderen hoe stress invloed uitoefent op het nemen van beslissingen en op het opslaan van herinneringen; tevens wordt gekeken naar de neurale codering van beide cognitive processen. Eerst een korte samenvatting over het nemen van beslissingen. Stel je voor dat je de keuze hebt tussen verschillende plaatsen om heen te gaan om voedsel of drinken te vinden. Bij de ene plaats bestaat een grote kans op voedsel, maar is de hoeveelheid voedsel per bezoek klein. De andere plaats heeft juist een lage kans op voedsel, maar dit komt dan ook in een grotere hoeveelheid. Als individuen de lage-kans optie prefereren, zijn zij bereid risico te nemen, terwijl de hoge-kans optie kenmerkend is voor een 'veilige' strategie. Er is weinig bekend over hoe stresshormonen (corticosteroiden) het nemen van risicovolle beslissingen beïnvloeden, en hoe zij het neurale substraat hiervan aansturen. Wij onderzoeken de hypothese dat stress een behoudend (veilig) keuzegedrag stimuleert, en dat dit merkbaar is in een veranderde codering van de waarde van verwachte beloningen in de orbitofrontale cortex, een gebied dat met affectieve keuze te maken heeft. Dit laatste aspect zullen wij onderzoeken door middel van zogenoemde /tetrode recordings/ in de OFC van ratten. Deze methode wordt veel toegepast in dit soort onderzoek bij ratten; het ongerief is gering tot middelmatig. Daarnaast zullen we, ook met tetrodes, bestuderen hoe stresshormonen processen beïnvloeden die met het opslag van herinneringen te maken hebben (specifiek: consolidatie en replay, d.i. het terugkeren van ervaringsspecifieke aktiviteitspatronen tijdens de slaap na de leerervaring). Dit gebeurt door zowel in de hippocampal als in de orbitofrontale cortex te meten. Wij verwachten dat deze studie een belangrijke bijdrage zal leveren aan de kennis over de neurale basis van de interactie van stress op beslissingen en op geheugen, en zal bijdragen aan het vinden van betere therapieen voor behandeling van stress- en angststoornissen, zoals posttraumatische stress stoornis (PTSD).

Werkprotocol

(Per experiment een apart protocol invullen dat bij het experiment in de dierverblijven aanwezig moet zijn.)

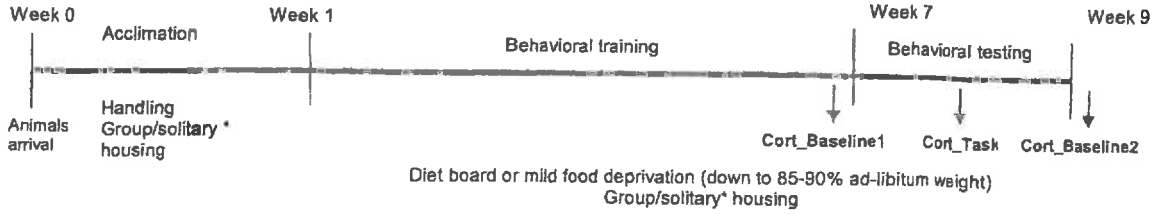
1 Doel van de proef

The broad aim of this series of experiments is the understanding of the role of stress on the neural coding of decision making and in regulating neural correlates of memory consolidation.

Pilots (phase 1): The optimization of behavioral tests for the study of offects of stress hormones on decision making and memory consolidation in rats;
Experiment (phase 2): Behavioral testing of decision making and memory consolidation during neural ensemble recordings in orbitofrontal cortex and hippocampus of rats.

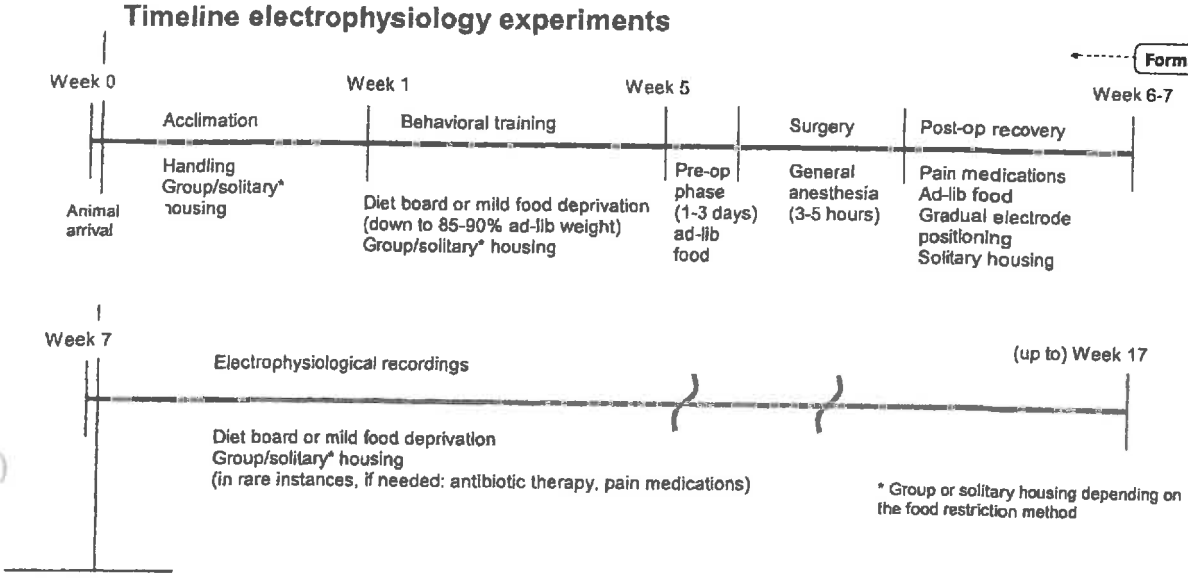
2 Welke handelingen/ingrepen worden er per dierproef verricht op het dier? Indien mogelijk tijdstippen van de ingrepen op een tijdsas weergeven.

Timeline behavioral pilot experiment



Diet board or mild food deprivation (down to 85-90% ad-libitum weight)
Group/solitary* housing

Timeline electrophysiology experiments



Formatted: Indent: Left: -1,8 cm

3 **Anesthesie.**
(techniek, middel, wijze van toediening, dosering: volledige beschrijving)

Formatted: Indent: Left: -1,8 cm

Surgery :
Inhalation: isoflurane 1-2% maintenance with (before induction) 0.01-0,05 mg/kg buprenorfine sc.

The rat's natural body temperature will be maintained by means of a closed loop thermal heating pad. The total duration of anesthesia will not be greater than 4-5 hours (in general, 3 hours).

End of experiment: Overdose Nembutal, 120 mg/kg .

4 *Pijnbestrijding.*
(middel, wijze van toediening, dosering; postoperatief: bij exp met wakkere dieren; etc)
To reduce pain and discomfort, we will administer buprenorfie (0.01-0.05mg/kg) before the surgery, and as an additional post-surgical care, animals will be rehydrated with 5 to 10 ml of subcutaneous isotonic saline. To prevent hypothermia, animals will be kept on thermal heating pads until complete recovery from anesthesia. Animals will be fed soft food until normal behavior is restored, then they will be put back on the usual diet. We will also use antibiotic treatment (baytril, see chapter 7 below) as anti-infection strategy.

5 Wie voeren welke handelingen onder 2,3 en 4 uit?

6 Beschrijf (geen kwalificatie van gering/matig etc) het te verwachten ongerief voor het dier (wat zie je aan het dier?), rekening houdend met factoren als behandeling, frequentie, tijdsduur, herhaling etc (eventueel per proefgroep)?
For pilot behavioral experiments :
Shaping: exposure to novel environments, getting accustomed to eating/drinking in those unusual environments (approx. 4 weeks) – we will check that animals do not show abnormal behavior, freezing, extreme activity, abnormal grooming, and that they eat normally in the experimental setup
Food-deprivation: Diet board or partial food deprivation, weight will be monitored constantly. (Approx. 11 weeks)
Corticosteroid injection: Animals will experience minor discomfort due to intraperitoneal injection of corticosteroids
For electrophysiology experiment:
Shaping: same as above (Approx. 4 weeks)
Surgery: placement in stereotaxic restraint (ear bars), head incision, craniotomy, electrode and micro-drive implant, under general anesthesia (isofluorane/buprenorphine or hypnorm/dormicum; about 3 hours; rectal temperature kept constant with a closed-loop heating system). The pedal reflex (or absence thereof) will be used as a measure of anesthesia level.
Post-operational stage: Animals will experience little to moderate discomfort after they have regained consciousness from anesthesia. Animals will be rehydrated and kept in warm environment. If no complications occur, normal behavior should be restored after 24-48 hours. Rats are able to tolerate the weight of the recording implant and compensate for its weight with the neck musculature. Normal rearing, locomotive behavior will be checked.
Experimental phase (i.e. the rat is performing behavioral task with implanted tetrodes in cranium): Micro-drive weight is easily bearable by rats, which can freely move and run (approx. 4-8 weeks).
Solitary housing: It is required to avoid rats damaging each other's implants. Animals can still see, hear and smell each other while in separate cages.
Food-deprivation: Diet board or partial (around 12 g chow a day). Weight will be monitored constantly (no less than 85% ad libitum weight). (Approx. 8-14 weeks)

7 Is er een kans op complicaties en/of bijkomende onbedoelde risico's van ongerief? Zo ja welke?

a) Death during surgery. This is a potential risk as rats are known to be very sensitive to overdose of anesthetics.
b) Insufficient recovery after the operation. In our experience with rats, this has not happened on previous occasions.
During surgeries, care is taken to operate in the most sanitary and sterile environment possible, according to common standards and applicable regulations for surgery on rodents. Still, infections may occur over time after surgery, inside and in the areas surrounding the craniotomy. These affect the grip of the anchoring screws on the skull, and may eventually cause the implanted device to detach, prematurely ending the experiment. To prevent implant rejection, tobramycin (3%) may be mixed to the first layer of dental cement used to fixate the implant. In rare instances, treatment with Baytril may be needed to combat post-surgery infections. In our hands ((Jackson et al. 2006); Jackson 2006, Lee et al. unpublished observations) as well as in other labs (e.g. Knierim, Redish and McNaughton labs), this greatly increases the mean lifetime of the implants after surgery, allowing a larger number of experimental sessions and a larger data yield, hence reducing the number of experimental animals needed. Care will be take to keep the waste of Baytril-treated animals separate from other animals. During the recovery period the areas surrounding the craniotomy will be regularly cleaned with betadine to reduce the risk of proliferation and propagation of bacteria.

8 Op welke indicatie worden de dieren voortijdig gedood? Wat moet er met de dode dieren gebeuren?

1) Permanent weight loss (max. 10 days) after surgery. If permanent weight loss is observed (<80% of free-feeding body weight, as determined before the experiment), the rats' food will be supplemented. If weight stays below 80% for several consecutive days in a row (up to 1 week), the rat will be sacrificed. Note that the maximum acceptable weight loss during the experiment is 15%. Weight loss higher than 15% will be counteracted by food supplements.
2) If pathogenesis without hope of recovery is determined. Visible signs of pathogenesis will be monitored such as infection, and condition of the fur. Behavior (grooming, sleeping, eating, exploring) will also be monitored for signs of abnormality.
3) When electrophysiological readings show that the hyperdrive is no longer firmly in place. This will be shown by a disruption of the normally stable background noise and an inability to stably record cells for a number of consecutive sessions. A recovery operation is then no longer possible.

Euthanasia will be applied when a rat is sick, as apparent in the general parameters. (see question 14.)
When an animal is found dead it will be kept in the fridge or freezer.

9 Wie moet er gewaarschuwd worden bij onverwachte gebeurtenissen?
(vermeld telefoonnummers ook voor de weekenden)

10 Wordt het dier na de proef gedood of hergebruikt?
Animals used for behavioral pilot studies will be made available for other experiments, or will be killed by overdose of pentobarbital, Animals used for electrophysiological recordings will be perfused with paraformaldehyde for subsequent brain histological preparations and electrode placement verification.

11 Wijze van termineren (volledige beschrijving).
Pilot study animals: those which are not reused will be euthanized by overdose of pentobarbital (120 mg/kg, i.p.).
Electrophysiology study animals: same as above, and, in addition, transcardial perfusion with paraformaldehyde.

12 Hoe ernstig schat u het cumulatieve ongerief voor het dier, rekening houdend met factoren als behandeling, frequentie, tijdsduur, herhaling etc. (evt per groep/experiment)?
Moderate.

13 Wat wordt gedaan om eventuele pijn, stress of ander ongerief te verminderen/ voorkomen?
Wie zijn hier verantwoordelijk?
Animals will be housed groupwise or in solitary housing. During the behavioral pilot the rats will be housed at least 3 per cage to avoid stress induced by the removal of a companion rat during testing sessions. If the rats are food deprived using the scheme described at 4.1.1 and the weight is not kept within expected parameters (E.g. one of the rats exceeds 90% of ad-libitum weight, or others drop below 85%), they will be moved from group to solitary housing. Rats in the diet board group will be group housed. Rats in the electrophysiology group will be single house to avoid damage to the implant and injury. For single housed animals cages will be arranged so that rats can still see and smell each other. The animals will be regularly handled to avoid stress. · will be responsible. Analgesic medications will be administered after surgery and in the post-operative days as needed.

14 Welke parameters en met welke frequentie moeten worden bijgehouden om het ongerief in te schatten en geef deze weer op een vel in de vorm van een matrix?
bv. Gewicht, eetlust, temperatuur, gedragskenmerken (bv manier van bewegen, afzondering, uiterlijke kenmerken (bv neus, bek, ogen, huid, haren ogen, houding), ademhaling, geboorte, nestgrootte zie o.a. code of practice: welzijnsbewaking)

Daily: weight, general aspect, fur, eyes, quantity, aspect of feces, fluid food consumption
Algemene parameters: Gewicht, Vuile ogen, Vuile neus, Doffe vacht, Haren overeind, Bolle rug, Diarrhee.

REFERENCES

Bechara A, Damasio H, Tranel D, and Damasio AR. Deciding advantageously before knowing the advantageous strategy. *Science* 275: 1293-1295, 1997.
Bechara A, Tranel D, Damasio H, and Damasio AR. Failure to respond autonomically to anticipated future outcomes following damage to prefrontal cortex. *Cereb Cortex* 6: 215-225, 1996.
de Quervain DJ, Roozendaal B, and McGaugh JL. Stress and glucocorticoids impair retrieval of long-term spatial memory. *Nature* 394: 787-790, 1998.
Dias-Ferreira E, Sousa JC, Melo I, Morgado P, Mesquita AR, Cerqueira JJ, Costa RM, and Sousa N. Chronic stress causes frontostriatal reorganization and affects decision-making. *Science* 325: 621-625, 2009.
Droste SK, de Groote L, Atkinson HC, Lightman SL, Reul JM, and Linthorst AC. Corticosterone levels in the brain show a distinct ultradian rhythm but a delayed response to forced swim stress. *Endocrinology* 149: 3244-3253, 2008.
Ernst M, Nelson EE, McClure EB, Monk CS, Munson S, Eshel N, Zarahn E, Leibenluft E, Zametkin A, Towbin K, Blair J, Charney D, and Pine DS. Choice selection and reward anticipation: an fMRI study. *Neuropsychologia* 42: 1585-1597, 2004.
Jackson JC, Johnson A, and Redish AD. Hippocampal sharp waves and reactivation during awake states depend on repeated sequential experience. *J Neurosci* 26: 12415-12426, 2006.
Ji D, and Wilson MA. Coordinated memory replay in the visual cortex and hippocampus during sleep. *Nat Neurosci* 10: 100-107, 2007.
Joels M. Functional actions of corticosteroids in the hippocampus. *Eur J Pharmacol* 583: 312-321, 2008.
Joels M, Pu Z, Wiegert O, Oitzl MS, and Krugers HJ. Learning under stress: how does it work? *Trends Cogn Sci* 10: 152-158, 2006.
Kepecs A, Uchida N, Zariwala HA, and Mainen ZF. Neural correlates, computation and behavioural impact of decision confidence. *Nature* 455: 227-231, 2008.
Kim JJ, Lee HJ, Welday AC, Song E, Cho J, Sharp PE, Jung MW, and Blair HT. Stress-induced alterations in hippocampal plasticity, place cells, and spatial memory. *Proc Natl Acad Sci U S A* 104: 18297-18302, 2007.
Lansink CS, Goltstein PM, Lankelma JV, McNaughton BL, and Pennartz CM. Hippocampus leads ventral striatum in replay of place-reward information. *PLoS Biol* 7: e1000173, 2009.
Liston C, Miller MM, Goldwater DS, Radley JJ, Rocher AB, Hof PR, Morrison JH, and McEwen BS. Stress-induced alterations in prefrontal cortical dendritic morphology predict selective impairments in perceptual attentional set-shifting. *J Neurosci* 26: 7870-7874, 2006.
Lupien SJ, and McEwen BS. The acute effects of corticosteroids on cognition: integration of animal and human model studies. *Brain Res Brain Res Rev* 24: 1-27, 1997.

McEwen BS. Physiology and neurobiology of stress and adaptation: central role of the brain. *Physiol Rev* 87: 873-904, 2007.
Mobini S, Body S, Ho MY, Bradshaw CM, Szabadi E, Deakin JF, and Anderson IM. Effects of lesions of the orbitofrontal cortex on sensitivity to delayed and probabilistic reinforcement. *Psychopharmacology (Berl)* 160: 290-298, 2002.
Pais-Vieira M, Lima D, and Galhardo V. Orbitofrontal cortex lesions disrupt risk assessment in a novel serial decision-making task for rats. *Neuroscience* 145: 225-231, 2007.
Porcelli AJ, and Delgado MR. Acute stress modulates risk taking in financial decision making. *Psychol Sci* 20: 278-283, 2009.
Roesch MR, Taylor AR, and Schoenbaum G. Encoding of time-discounted rewards in orbitofrontal cortex is independent of value representation. *Neuron* 51: 509-520, 2006.
Rogers RD, Owen AM, Middleton HC, Williams EJ, Pickard JD, Sahakian BJ, and Robbins TW. Choosing between small, likely rewards and large, unlikely rewards activates inferior and orbital prefrontal cortex. *J Neurosci* 19: 9029-9038, 1999.
Roitman JD, and Roitman MF. Risk-preference differentiates orbitofrontal cortex responses to freely chosen reward outcomes. *Eur J Neurosci*.
Sadowski RN, Jackson GR, Wieczorek L, and Gold PE. Effects of stress, corticosterone, and epinephrine administration on learning in place and response tasks. *Behav Brain Res* 205: 19-25, 2009.
Sakai RR, McEwen BS, Fluharty SJ, and Ma LY. The amygdala: site of genomic and nongenomic arousal of aldosterone-induced sodium intake. *Kidney Int* 57: 1337-1345, 2000.
Seo H, and Lee D. Orbitofrontal cortex assigns credit wisely. *Neuron* 65: 736-738.
Stamp JA, Mashoodh R, van Kampen JM, and Robertson HA. Food restriction enhances peak corticosterone levels, cocaine-induced locomotor activity, and DeltaFosB expression in the nucleus accumbens of the rat. *Brain Res* 1204: 94-101, 2008.
van Duuren E, Escamez FA, Joosten RN, Visser R, Mulder AB, and Pennartz CM. Neural coding of reward magnitude in the orbitofrontal cortex of the rat during a five-odor olfactory discrimination task. *Learn Mem* 14: 446-456, 2007.
van Duuren E, Lankelma J, and Pennartz CM. Population coding of reward magnitude in the orbitofrontal cortex of the rat. *J Neurosci* 28: 8590-8603, 2008.
van Duuren E, van der Plasse G, Lankelma J, Joosten RN, Feenstra MG, and Pennartz CM. Single-cell and population coding of expected reward probability in the orbitofrontal cortex of the rat. *J Neurosci* 29: 8965-8976, 2009.