A time to remember

Biemans, Barbara Agatha Maria

Chapter 1

Introduction

Contents

I. THE CIRCADIAN SYSTEM, LEARNING AND MEMORY

Circadian variation in performance

The ability to acquire a task can vary with time of day. In humans, performance in many learning tasks varies across the 24 hours. The best results are often obtained during morning hours (Dijk *et al.*, 1992; Kraemer *et al.*, 2000), but the time of maximal performance varies between chronotypes, i.e. morning or evening types (Intons-Peterson *et al.*, 1998; Intons-Peterson *et al.*, 1999). Charles Stroebel found that in humans and day-active monkeys, neurosis-fear behaviour towards an unavoidable frightening object is learnt most rapidly during the early morning hours (Stroebel, 1965). One might predict that nocturnal (night active) animals perform better during the dark phase. Apparently, this is not always the case. Some studies have shown that there is a day-night difference with better initial acquisition during the day, the inactive period for nocturnal animals. For example, Davies *et al.* (1973) demonstrated a 24-hour rhythm in passive avoidance behaviour. In groups of rats trained and tested in 4-hourly intervals throughout a 24-hour cycle, retention (measured 48 hours after training) was highest in the middle of the day, and lowest in the middle of the night. This could of course be due either to better learning or to better retrieval. Chaudhury and Colwell (2002) also found that mice consistently acquire tone-cued fear conditioning better during the day. Explanations have been sought in the idea that an animal may be extra alert when woken up, since this is more "disturbing" than testing them during their active period. Opposite results were found in C57BL/6J mice, displaying enhanced context-dependent fear conditioning during their active phase (Valentinuzzi *et al.*, 2001). Stroebel (1965) found the speed of emotional conditioning in rats to vary with circadian phase, with peak acquisition rates at the transition of light to dark (onset of activity). Finally, social memory is not at all influenced by circadian time (Reijmers *et al.*, 2001). Thus, the results appear to depend on species and on the employed learning paradigms.

Periodic memory deficits

In 1957, Leon Kamin reported a decrement in memory of rats when tested 0.5, 1 or 6 hours after an incompletely learnt two way active avoidance (shuttle box) response. Twenty-four hours and 19 days after learning the response was indistinguishable from the group tested immediately after learning. Kamin interpreted this phenomenon as the sum of action of two independent processes: a forgetting process and an "incubation" (increase of fear) process (Kamin, 1957; 1963). After careful examination (sampling every 0.25 hours until 1.5 hours after learning), the locus of

the maximal "Kamin effect", was found to be 1 hour after learning (Denny and Ditchman, 1962). Klein and Spear (1970) showed that prior active avoidance training interferes with new passive avoidance learning at the intervals directly after and 24 h after training (when the memory for the first task is high). In contrast, the acquisition of the new passive response was optimal (comparable to that of naïve rats) at the intermediate (Kamin) intervals at 1 and 4 h after training of the original response, when memory for the original conflicting (active) task was low. Their results further indicated that differences in memory retrieval, not fear, mediate the Kamin effect. The authors concluded that the effect might best be explained by state-dependent retrieval (Klein and Spear, 1970). Meanwhile, Stroebel (1967) had shown that acquisition of a conditioned emotional response (CER) was most rapid when training occurred always at the same time of day, than when presented at random times. In that same study, extinction was most rapid with training and extinction trials always occurring at the same time of day. Stroebel also discovered that the strongest response to unavoidable fear (caused by footshocks) shifted together with a 12-hour phase shift in light dark cycles, demonstrating a link to a biological timing system.

Few years later, Frank Holloway and Richard Wansley embarked upon an enterprise that later turned out to be the major breakthrough in the issue, and indeed of great significance to the field of learning and memory at large. Evidently, the memory "dip" reported by Kamin was not a single event reflecting a transitory retention impairment, but a long-lasting phenomenon recurring at periodic intervals! In a one-trial passive shock procedure, they demonstrated that independent groups of rats displayed higher retention scores immediately and at successive multiples of 12 hours after training than at 6 hours, or at multiples of 12 hours from this 6-hour interval (Holloway and Wansley, 1973a). The periodicity was present throughout three complete day-night cycles following training. Unshocked control groups did not show any differences in latencies to enter the dark compartment. The possibility of a simple circadian or diurnal factor as the direct source of memory oscillations was subsequently ruled out by another study: Holloway and Wansley (1973b) showed that the deficit pattern is independent of the time of training (Fig.1). With four training times at 6-hour intervals, both a passive and an active shock avoidance paradigm yielded maximal memory retrieval always at 24 hours after training. That the pattern is not a 12-h periodicity was disclosed by the higher cut-off criterion in this study compared to previous studies. Troughs in retention occurred at 6, 18 and 30 hours after single-trial passive shock avoidance when testing groups of rats every 2 hours up to 36 hours after training (Wansley and Holloway, 1976).

Subsequently, periodic memory deficits were also demonstrated in appetitively motivated learning tasks. Deficits at 6, 18 and 30 hours after training were present in the latency to find a water sprout in a simple "irregular maze" paradigm (Wansley and Holloway, 1975), suggesting some degree of generality across tasks. Hunsicker and Mellgren (1977) designed a combined appetitive - aversive paradigm. They varied the interval between a single pre-exposure to sugared water in the passive shock box and subsequent one-trial passive avoidance training, but retained the interval between passive avoidance training and testing at 24 hours, to control for motivational factors. Here, deficits were present at 3, 6, 15, and 18 hour intervals, and good retention at 0.25, 9, 12, and 24 hours. Other investigators did not find a Kamin effect in a completely (Bintz *et al.*, 1970) and incompletely (Hablitz and Braud, 1972) learnt strictly appetitive response. Avoidance learning in a discriminative Y-maze yielded only a single decrement at 1 hr after training (Caul *et al.*, 1974). Some of these descepancies could be explained by the use of multiple instead of single trainings procedures (see also chapter 9).

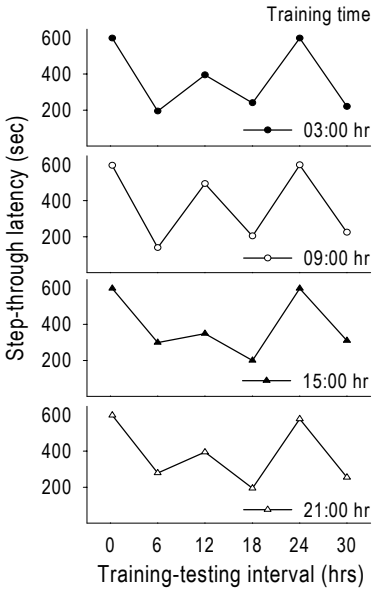

Figure 1. Memory retention at multiple training-testing intervals in a passive shock avoidance paradigm. Training took place at four diferent times of day. Adapted from: Holloway & Wansley, 1973b.

To account for retention periodicities, theories have been based on the idea of some biological factor modulating retention performance. Holloway and Wansley postulated that the results could be explained by state-dependent learning, in which a pre-training rhythmic process may influence or define the state of the animal, and shifts away from that state may produce an internal dissociation (Holloway and Wansley, 1973a,b cf Klein and Spear, 1970). As an alternative, they also suggested that the exposure to the shock might trigger or reset a rhythmic or oscillatory variable, which would then interfere with retention. Although a strong aversive stimulus is absent in the appetitive tasks, these tasks also contain stressful components, such as deprivation treatment and the reinforcement event.

In a recent study by Ralph and co-workers (2002), hamsters were trained in a conditioned place preference task at two times in the circadian cycle, during the dark and during the light phase. They found that the hamsters only displayed a clear preference for the reinforcing stimulus, a couloured chamber with a running wheel, at the time at which they had been trained. No preference was found at other times,

even when these times coincided with the subjective night, when a high spontaneous preference for wheel running is expected. In contrast, Chaudhury and Colwell (2002) have recently conducted an extensive study in house mice, training and testing individual mice repeatedly in two fear conditioning paradigms. Their mice showed maximal memory for both tasks always during the subjective day, irrespective of time of training, mouse strain, or light-dark regime. This is seemingly at odds with the rat studies. I will return to this issue in chapter 9.

Manipulation of the circadian system

Since the pattern in memory deficits is suggestive of a biological clock system as underlying factor, it has prompted researchers to study memory retention after tampering with the circadian system in a variety of ways. One of these approaches was lesioning of the at that time freshly discovered generator of circadian rhythms (Stephan and Zucker, 1972; Moore and Eichler, 1972), the suprachiasmatic nucleus of the hypothalamus (SCN). Stephan and Kovacevic (1978) lesioned the SCN of rats and let them undergo the Holloway and Wansley passive shock protocol. Performance of the SCN lesioned rats at 18 and 30 hours after training was as good as those in the 24-hour interval group, whereas the control and sham lesioned groups again displayed significant retention deficits at these times. Acquisition was not affected in lesioned animals: the pre-shock latencies were comparable for all groups (Stephan and Kovacevic, 1978). Apparently, the SCN exerts some specific suppressing effect on memory retention.

Another logical step to demonstrate the control of the circadian system over memory oscillations was to induce phase shifts, and see whether this would affect memory deficits. Davies *et al.* (1974) were the first to do so, and found detrimental effects on passive shock avoidance retention after a 6 hour forward phase shift in rats. In most subsequent studies, amnestic effects of phase shifts were confirmed for rats (Tapp and Holloway, 1981; Fekete *et al.*, 1985) and mice (Stone *et al.*, 1992). Devan *et al.* (2001) subjected rats to five consecutive days of 3-hour phase shifts during training in the water maze and found memory impairments 15 days later. Social memory however, was not affected by phase shifts in rats (Reijmers *et al.*, 2001).

Phase shifts induced by travelling across time zones ("jet lags") can have mild, to severe adverse effects on attention, reaction time, and concentration in humans (Hauty and Adams, 1966a,b; Klein *et al.*, 1972; Conroy, 1972). Wright *et al.* (1983) have shown decrements in cognitive performance after a transmeridian flight, possibly due to sleep disturbances. Memory impairments were found in airline cabin crew exposed to chronic jet-lag (4 years of transmeridian flying experience) in case of

short intervals between flights, but not in case of longer intervals. Longer intervals (>14 days) would be sufficient to allow recovery of circadian rhythms to re-adjust (Cho *et al.*, 2000). Thus, findings in animal studies with respect to memory impairment as a consequence of phase shifts seem consistent with those in humans.

Taken together, considerable evidence is available for a substantial link of the circadian pacemaker to learning and memory processes, but the precise mechanism of action remains unclear so far.

II. AGEING

Ageing of the circadian system

Circadian rhythmicity is disrupted with ageing at all organisational levels. Notable changes take place in behaviour and physiology, for example altered day-night distribution of activity and decreased amplitude of corticoid hormones and body temperature. (For reviews see: Ingram *et al.*, 1982; Van Gool and Mirmiran, 1986a; Aschoff, 1994; Turek *et al.*, 1995; Van Someren, 1997; Weinert, 2000). The most prominent and consistently found feature is the age-associated decrease of rhythm amplitude and disruption of normal circadian patterning (fragmentation). Aged humans, especially demented elderly, suffer from severe changes in rest-activity behaviour: increased daytime napping and waking and increased restlessness during the evening and night (Prinz *et al.*, 1982; Allen *et al.*, 1983). In fact, the major problem in caring for demented patients is that their rest-activity rhythm is so disturbed that they need round the clock care (Swearer *et al.*, 1988; Pollak and Perlick, 1991; Gallagher-Thompson *et al.*, 1992). Here, some changes in overt and covert rhythms will be outlined.

Behaviour

Humans and animals have reduced amplitudes of body temperature (Yunis *et al.*, 1974; Weitzman *et al.*, 1982; Czeisler *et al.*, 1992; Satinoff, 1998; McDonald *et al.*, 1999) and hormonal rhythms, e.g.: corticosterone (Paris and Ramaley, 1974; Nicolau and Milcu, 1977), serum testosterone (Simpkins *et al.*, 1981), thyroid stimulating hormone (Klug and Adelman, 1979), and melatonin (Reiter *et al.*, 1981). Activity rhythms in rats (Slonaker, 1912), hamsters (Scarbrough *et al.*, 1997; Davis and Viswanathan, 1998), and mice (Welsh *et al.*, 1986a; Valentinuzzi *et al.*, 1997), as well as the sleep-wake rhythm (Van Gool and Mirmiran, 1986b) are similarly affected. Extended activity periods (α) in combination with lower amplitudes leads to damped rhythms. Increased fragmentation and altered light-dark

distribution of activity occurs (Slonaker, 1912; Wax, 1975; Peng *et al.*, 1980; Van Gool and Mirmiran, 1983; Van Gool and Mirmiran, 1986b; Penev *et al.*, 1997; Antoniadis *et al.*, 2000), even up to complete arrhythmicity (Peng and Kang, 1984; Satinoff *et al.*, 1993; Walcott and Tate, 1996; Weinert and Weinert, 1998). The stability of free-running rhythms is compromised in mice (Weinert and Weiss, 1997), hamsters (Aschoff, 1994), and humans (Wever, 1992). Free-running periods (τ) of activity rhythms have been reported consistently shorter in rats (Richter according to Aschoff, 1994; Rietveld *et al.*, 1985; Van Gool *et al.*, 1987; Witting *et al.*, 1994) and hamsters (Pittendrigh and Daan, 1974; Morin, 1988; Rosenberg *et al.*, 1991; Watanabe *et al.*, 1995). For aged mice, predominantly longer (Davis and Menaker, 1981; Welsh *et al.*, 1986b; Valentinuzzi *et al.*, 1997; Mayeda *et al.*, 1997), but also shorter (Pittendrigh and Daan, 1976) periods have been found.

With respect to responses to environmental cues, a loss of sensitivity and altered responses to photic and non-photic cues time cues have been reported, measured by phase shifting induced mainly by light pulses (Rosenberg *et al.*, 1991; Aschoff, 1994; Provencio *et al.*, 1994; Zhang *et al.*, 1996). Also, altered phase relationships, especially phase advances were reported in elderly humans: for the sleep-wake cycle (Reilly *et al.*, 1997; Duffy *et al.*, 1998), body temperature (Monk *et al.*, 1995), and hormones (Van Cauter *et al.*, 1996). Old hamsters (Zee *et al.*, 1992; Scarbrough *et al.*, 1997) and mice (Weinert and Weinert, 1998) have been shown to start activity earlier in the day than young animals, although delayed of activity onset has been described (Valentinuzzi *et al.*, 1997) as well. A phase advance of body temperature compared to activity was found by Yunis *et al.* (1974) in old rats and mice. Resynchronisation to the Zeitgeber can be slower in old rats (Buresova *et al.*, 1990), mice (Valentinuzzi *et al.*, 1997) (only with phase advances), and humans (Reilly *et al.*, 1997).

SCN neurochemistry

Changes in behaviour may be caused directly by decreased neuronal integrity of the SCN, or by loss off coupling between pacemaker cells and their output. In an electrophysiological study by Satinoff *et al.* (1993), cultured SCN cells, taken from aged rats, showed damping of peak neuronal firing rate in vitro compared to young rats' cells. However, not all rats with aberrant neuronal firing (arrhythmic, damped or other peak times) displayed loss of circadian rhythms in body temperature, drinking or locomotor activity, and vice versa. This indicates that behavioural entrainment can be achieved in the absence of a neuronal rhythm. Damped neuronal firing has also been observed in hamsters (Watanabe *et al.*, 1995). A loss of peptidergic neurones containing the neurotransmitter vasopressin has been reported in humans (Swaab *et al.*, 1985), rats (Roozendaal *et al.*, 1987; Lucassen *et al.*, 1995),

and voles (Van der Zee *et al.*, 1999a). Also, vasoactive intestinal peptide producing neurones in rats (Chee *et al.*, 1988), and mRNA in hamsters (Duncan *et al.*, 2001) and rats (Kawakami *et al.*, 1997; Krajnak *et al.*, 1998) are reduced with age. The total number of SCN neurones does not change with ageing, at least not in rats (Peng *et al.*, 1980; Roozendaal *et al.*, 1987; Madeira *et al.*, 1995). In humans, Swaab and co-workers (1985) found a significant reduction in SCN cell number in aged subjects, and even more so in Alzheimer's patients.

Hence, age-related alterations are found within the SCN. Transplantation of SCN-grafts of young hamsters into aged hamsters restored circadian rhythms (Hurd *et al.*, 1995; Viswanathan and Davis, 1995). The same occurred with foetal tissue in rats (Li and Satinoff, 1998). This clearly implicates the SCN in the age-related changes in behavioural output systems. Some of the degenerative changes of the circadian system with old age can be overturned. The 'use it or loose it' concept of Swaab (1991) states that activation of nerve cells (for example by hormones or transmitters), may prevent degeneration and may even restore their function. He predicted that increased input to the circadian system could relieve some of the symptoms described. Alzheimer's patients are exposed to much less environmental light compared to age-matched controls (Campbell *et al.*, 1988). In rats, high light intensities reversed age-related amplitude decrease of sleep-wake patterns (Witting *et al.*, 1993). This now has proven to be an effective therapy to reduce rest-activity disturbances in humans as well (Van Someren *et al.*, 1997; Van Someren *et al.*, 2002a). At the neuronal level, increased light input counter-acted the age-related decrease in vasopressin cells in aged rats (Lucassen *et al.*, 1995).

Age-related decline in memory

An important goal in cognitive neurosciences is to characterise neuronal mechanisms of memory function. The gradual loss of cognitive capacity seriously affects the quality of life in many elderly. Severe amnesia as seen in demented patients is not only debilitating, but can have great emotional impact, both on the patient and those close to him or her. It is therefore vital that therapies are sought to reverse, at least to some extent, the adverse effects of ageing on cognition. Experimental studies in animals have received much and still growing attention. Rodents, especially rats, are widely used because of the many similarities to humans with respect to brain processes, in particular the pathways involved in cognition. Rats are particularly popular because they are intelligent (Davis, 1996) and thus able to learn very complicated tasks. This fact, combined with their relatively short life span (2-4 years) and low rearing costs, has led to a wide variety of available ageing models (Sprott, 1991) and a choice of learning tasks, discriminating between, and tapping on

different memory systems in the brain. One has to choose carefully the task that suits one's purpose best, taking into account strain (and supplier!), age, gender, life span, pathologic conditions, required training time, etc. Strain differences definitely play an important role in the outcome of any study (Ingram *et al.*, 1994; Van der Staay and Blokland, 1996). More recently, mice are becoming popular experimental models because of the advantages of genetic modification.

Many confounding factors exist that imply memory impairments but can be attributed to other, non-cognitive, age-related factors (for reviews, see: Barnes, 1990; Masoro, 1991; Andrews, 1996; Van der Staay, 2002). For example, a task like the water maze, in which subjects are trained to locate a submerged platform in a swimming pool using extra-maze cues, requires very good motor capacities. This can reveal inferior performance by aged rats that is merely due to exhaustion or poor motor abilities.

Behaviour

Detrimental effects of ageing were demonstrated across species in (almost) all studied cognitive tasks so far. Age related impairments in memory function are commonly reported in all types of learning tasks in rodents (reviews: Barnes, 1990; Muir, 1997), although the severity of the deficit is task dependent. Spatial memory is particularly susceptible to ageing effects. Humans (Evans *et al.*, 1984), other primates, and rodents are similar in the sense that with increasing age tasks with a strong spatial component give them more difficulties (for reviews see): (Barnes, 1987; Barnes, 1990). Impaired memory has been shown in the simple and complex T-maze (Goodrick, 1968; Winocur, 1984; Lowy *et al.*, 1985; Goldman *et al.*, 1987; Ingram, 1988; Meyer *et al.*, 1998), radial mazes (Barnes *et al.*, 1980; Van Gool *et al.*, 1985; Gallagher *et al.*, 1985), and water maze (Morris, 1981) even after correcting for swim speed, or when taking swim distance as a measure of performance (Gage *et al.*, 1984; Rapp *et al.*, 1987; Lindner and Gribkoff, 1991; Hebda-Bauer *et al.*, 1999; Van der Staay, 2000). Ageing also disrupts holeboard learning, another spatial task (Barnes *et al.*, 1980; Rigter *et al.*, 1984; Van der Staay *et al.*, 1990). One of the first brain structures to show age-related changes is the hippocampal formation, crucial for the formation of long term memory and for spatial mapping (O'Keefe and Nadel, 1978). This could explain the specific spatial vulnerability (Barnes *et al.*, 1987; Gallagher, 1997).

Classical (fear) conditioning (Houston *et al.*, 1999; Doyere *et al.*, 2000), place preference (Antoniadis *et al.*, 2000) and eye-blink conditioning (Woodruff-Pak, 1988; Knuttinen *et al.*, 2001) are also affected by age. Shock motivated tasks are widely used for examining the effect of age on acquisition, retention and extinction. Passive, or inhibitory avoidance is suppressed (Sprott, 1972; Bartus *et al.*, 1980;

Lippa *et al.*, 1980; Dean *et al.*, 1981; Kubanis *et al.*, 1982; Martinez, Jr. and Rigter, 1983), as well as (one- or two-way) active shock avoidance conditioning (Freund and Walker, 1971; McNamara *et al.*, 1977; Fuchs *et al.*, 1986; Stone and Gold, 1988; Van der Staay and Blokland, 1996; Ambrosini *et al.*, 1997; Spangler *et al.*, 1997). In all these tasks, the degree of age-related deficits varies across, and even within laboratories, but the general pattern is definitely one of cognitive deterioration.

Neurochemistry

Ageing is associated with a decline in neural pathways. Important findings in this respect were the decrease of choline acetyltransferase (ChAT) activity in the post-mortem brains of demented patients (Bowen *et al.*, 1976; Davies and Maloney, 1976) and the loss of cholinergic neurones in the nucleus basalis of Meynert (nbM) of the basal forebrain (Whitehouse *et al.*, 1981; 1982). The subsequent landmark study reporting a correlation between the Alzheimer's disease patients' mental state scores and cholinergic abnormalities in their post-mortem brains (Perry *et al.*, 1978) led to Bartus' "cholinergic hypothesis" of dementia (Bartus *et al.*, 1982). It attributes the learning and memory deficits in the aged, demented, and AD patients principally to a decline of cholinergic systems in the basal forebrain. This in turn stimulated a large number of animal studies employing cholinergic blockade or lesions of the basal forebrain cholinergic system (for reviews: Olton and Wenk, 1987; Blokland, 1996; Muir, 1997; Sarter and Bruno, 1998). Aged animals demonstrating memory impairments also suffer from neuronal loss and atrophy of surviving cholinergic neurones in the basal forebrain (Koh and Loy, 1988; Markram and Segal, 1990).

Although the body of evidence from experimental and descriptive studies in favour of a crucial function for the cholinergic system in cognition is substantial, the ageing process without doubt involves more than a single transmitter system. For example, other studies have shown the involvement of glutamatergic systems in learning and memory (for reviews: Ingram *et al.*, 1994; Gallagher, 1997). Determining the (relative) contribution and precise role of each system will remain the subject of investigation for many years.

The suprachiasmatic nucleus (SCN) is a small bilateral nucleus at the base of the anterior hypothalamus. It is easily distinguishable from the surrounding hypothalamic area because of its densely packed cell-structure. The SCN receives retinal information about ambient light conditions directly via the retino-hypothalamic tract, the main entraining pathway. Light is the most important and strongest Zeitgeber, by which rhythms in behaviour and physiology are entrained to the 24-hour cycle that is imposed by the rotation of the earth. Rhythms "freerun" with an endogenous period (τ) of about 24 hours (circa-dian: about-a day (dies)) in the absence of a Zeitgeber, and these self-sustained oscillations can continue for years. A brief overview of SCN anatomy and physiology is presented here, mainly based on (review) articles by (Stephan *et al.*, 1981; Watts *et al.*, 1987; Watts and Swanson, 1987; Buijs *et al.*, 1996; Van Esseveldt *et al.*, 2000).

The SCN of rats and mice contains approximately 10.000 neurones on each side. Individual SCN cells synthesise one or more of the following neuroactive substances: glutamate decarboxylase (GAD), γ-aminobutyric acid (GABA), arginine-vasopressin (AVP), vasoactive intestinal peptide (VIP), gastrin releasing peptide (GRP), somatostatin (SOM), substance P (SP), calretinin, bombesine, neurotensin, enkephaline (ENK), calbindin, angiotensin II. The quantity, distribution, and co-localisation of these transmitters depend heavily on species. This points towards species-specific roles for the various neuroactive substances. Peptide level is always a snapshot impression reflecting the balance between on the one hand an increase by *de novo*-synthesis, and on the other hand the dwindle due to degradation or release. Most, if not all cells are GABAergic, and GABA is therefore co-localised with many of the neuropeptides. GABA is now considered the most important neurotransmitter in the SCN (Moore and Speh, 1993). It exerts a tonic inhibitory action over the day, with apparent desinhibition during the subjective day, possibly effected by an interaction of GABA with other neuroactive substances. Subdivisions of the SCN can be based on functional aspects (e.g. input versus output), morphology (cell size and packing density, dendritic arbourisation, synaptic contact), or neurotransmitter content. The use of various terminologies by researchers depends on custom or goal, but the described areas largely overlap. The dorsomedial part of the SCN, also termed shell (Moore, 1996), contains mainly AVP and SOM, both of which remain rhythmic under constant light conditions (DD). The ventrolateral part or core mainly contains VIP and GRP, that are rhythmically expressed under LD conditions, but lose rhythmicity in constant darkness.

With the exception of VIP, peptide expression peaks during the day when electrical multiple unit activity of SCN neurones is also high, and is lower during the night. VIPergic neurones receive dense synaptic input from VIP and SOM neurones, whereas AVP neurones receive substantial innervation from VIP neurones in the core, and from AVP and SOM neurones in the shell. Fibres extending outside the SCN contain mainly VIP and AVP, originating from core and shell, respectively. GRP and SOM fibres remain essentially within the SCN. Generally, the smaller neurones in the shell have limited dendritic arbourisation and tend to extend beyond the border of the SCN. The larger core neurones have processes that are extensively branched, and form a dense axonal plexus within this subdivision. Neuronal interconnectivity is more pronounced in the core than in the shell, and shell-to-core projections are sparse, in contrast to intense core-to-shell projections. These anatomic characteristics of core and shell make them suitable for their respective input and output functions: the core is mainly concerned with the entrainment of the circadian system to the LD cycle, and the shell is assumed to be involved in the generation and coordination of overt rhythms.

The SCN receives excitatory glutamatergic input through the RHT from the retina, neuropeptide-Y (NPY) input from the intergeniculate leaflet (IGL) of the lateral geniculate nucleus through the geniculo-hypothalamic tract (GHT), and serotonergic (5-hydoxytryptamine (5-HT)) input from the raphe nuclei. All these projections synapse almost exclusively onto core neurones, and are involved in the direct entrainment to the LD cycle, and in indirect modulatory feedback on the synchronisation process. Moderate input to the shell alone originates from the cortex, basal forebrain, hippocampal formation and hypothalamus. The SCN sends its majority of efferent fibres to the subparaventricular zone (sPVZ) of the hypothalamic paraventricular nucleus (PVN), the starting point of the hypothalamo-pituitary-adrenocortical axis (HPA). Core and shell have segregated their input to the sPVZ, i.e. the core projects more to the lateral, and the shell to the medial sPVZ. Fairly dense projections connect to other hypothalamic areas: the dorso- and ventromedial hypothalamic nuclei (DMH, VMH), the medial preoptic area (MPO), and retrochiasmatic area. A substantial projection runs dorsally to the midline thalamus, in particular the paraventricular nucleus of the thalamus (PVT). The PVT innervates multiple limbic structures, such as the amygdala and nucleus accumbens (NAc), as well as cortical regions including the medial prefrontal cortex (mPFC). Apart from the SCN, the PVT receives input from the sPVZ, retina and IGL. In turn, the PVT projects back to the SCN, both to core and shell (Moga *et al.*, 1995). Many of these projections contain GABA with AVP or VIP, and they lay the basis for the range of behavioural and physiological functions under the control of the circadian pacemaker.

Ageing of the circadian system and memory

Severe learning and memory impairments and circadian disorganisation thus simultaneously occur with ageing, and especially in Alzheimer's dementia. Despite this, few researchers have yet addressed the question: "can a decrease in circadian organisation lead to the memory impairments found with ageing?". Bright light therapy has proven to be successful in increasing the stability of the rest-activity rhythm and reduce sleep-wake cycle disturbances in demented elderly (for reviews: Van Someren *et al.*, 1999; Van Someren, 2000). Effects of bright light on cognitive disturbances in Alzheimer-type dementia were found, in combination with resynchronisation of the body temperature rhythm (Graf *et al.*, 2001) or improvement of the sleep wake rhythm (Ito *et al.*, 1999). However, in these studies, rhythm improvement has not been suggested as a potential causative factor in enhancement of cognitive function, but merely as a simultaneous phenomenon.

Stone (1989) has suggested a role for circadian function in age-related memory impairments, putting together literature on the separate topics, while focusing on (paradoxical) sleep disturbance. His lab found that fragmented paradoxical sleep accompanies memory impairments in old rats, and that one measure of paradoxical sleep, bout duration, correlated significantly with retention scores in rats with lesions of the nucleus basalis magnocellularis (NBM) (Stone, 1989). Winocur and Hasher (1999) tested delayed alternation and inhibitory avoidance early and late in the night in young and aged rats. Only aged rats were influenced by time of day: they performed better in the beginning of the night, but always worse than the young ones. Finally, Antoniadis *et al.* (2000) performed a place preference study in aged hamsters. They assigned the hamsters to two groups, characterised by consolidated and fragmented rhythms. The aged hamsters with consolidated rhythms outperformed the fragmented hamsters in a context conditioning task, and activity amplitude was significantly associated with preference score.

Together, these studies provide the first answers to the intriguing and significant question about the role of the circadian system in the age-related memory decline. This thesis describes our efforts to further elucidate the matter.

Scope of the experiments

The main question leading to this research project was to what extent, if at all, ageing of the circadian system contributes to the age-related decline in memory function. This question can be subdivided into two parts:

1. In what fashion does the circadian pacemaker (SCN) interact with the process of learning and memory?

2. Does ageing affect the circadian modulation of memory at the behavioural and neurochemical level?
I have carried out several experiments with young and aged rodents. The first aim was to study whether memory oscillations are endogenously regulated, and to get a grip on the neurochemical processes in the SCN that may play a role in the modulation of memory. Secondly, I have studied aged individuals to determine whether alterations in circadian behaviour and mnemonic processes are present, and whether they are accompanied by altered neuropeptide dynamics in the SCN. The results and conclusions of these experiments are described in this thesis.

III. OUTLINE OF THE THESIS

This dissertation is organised in two parts. The first part "SCN and Memory Processes" describes experiments carried out with young rodents to examine several aspects of memory processes and the resulting changes in the SCN neuropeptides. **Chapter 2** describes a basic experiment. It addresses a question which should perhaps have been asked 30 years ago: whether periodic memory deficits require the presence of an LD cycle. Only if they persist in constant light conditions, it is reasonable to attribute them to an endogenous rhythm. This chapter also asks whether vasopressin, one of the major output systems of the pacemaker, plays a part in generating the fluctuations under constant light conditions. For this, Wistar, Long Evans, and vasopressin deficient Brattleboro rats were used. **Chapter 3** further explores the role of SCN-vasopressin in house mice of selected lines, that differ in basal vasopressin levels, stress response and anxiety, when presented with a stressful learning task. **Chapter 4** concludes the first part by focusing on the muscarinic acetylcholine receptor system of the SCN after active and passive shock avoidance training, as the cholinergic system is highly involved in learning and memory processes. Both the time course and the facet of fear conditioning responsible for alterations in receptor dynamics are investigated in adult Wistar rats.

The second part deals with "Ageing of the Circadian System and Memory". **Chapter 5** provides elementary information on age-effects in the circadian behaviour of rats and mice. It features Wistar Unilever, Fischer 344 × Brown Norway hybrid (F×BN) rats, and CBA/ca house mice. **Chapter 6** addresses the important and basic question whether memory oscillations persist when the same individual is tested repeatedly. This is a methodologically important issue, as all the classical experiments have painstakingly avoided testing individuals more than once, and have thereby used very large numbers of animals. Applying multiple testing, we could ask whether memory oscillations are influenced by a declined circadian system

with age. Young and aged F×BN rats were used here in a passive shock avoidance paradigm under constant light conditions. **Chapter 7** reports an increase in basal levels of SCN somatostatin and substance P immunoreactivity in aged Wistar rats compared to young ones. This contrasts with most other neuropeptides that are found to decline with ageing, such as vasopressin. Following up on Chapter 4, **Chapter 8** explores whether the cholinergic system, known to be drastically affected by ageing, is still able to respond to stress in aged rats (with a severely impaired circadian organisation of behaviour), as it does in young rats. Vasopressin was also studied in these Wistar Unilever rats, since it was found to be affected in previous studies (Chapter 2 & 3). Finally, **Chapter 9** summarises the results and conclusions, and discusses them in the framework of existing literature. It makes up the balance of what we have learnt so far, and what remains to be resolved to understand the role of the circadian system in learning and memory function.