DEHYDROEPIANDROSTERONE AND MEMORY

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List of abbreviations:

ANI, anisomycin

DHEA, dehydroepiandrosterone

DHEAS, dehydroepiandrosterone Sulphate

GABA, γ-aminobutyric acid

IMMP, intermediate medial mesopallium

i.c.v., intracerebroventricular

i.p., intraperitoneal

MeA, methylanthranilate

NMDA, N-methyl-D-aspartate

SCO, scopolamine

σ, sigma

Sts, steroid sulphatase

s.c., subcutaneous

1. Introduction

As its sulphate ester, dehydroepiandrosterone (DHEA) is the major circulating steroid hormone in the young adult primate and is secreted from the cortex of the adrenal gland. This circulating dehydroepiandrosterone sulphate (DHEAS) serves as pool of the free steroid DHEA, following uptake then desulphation by the steroid sulphatase (Sts) enzyme in various tissues (Reed et al. 2005; Miller 2009; Hobkirk 1985). Secretion of DHEAS by the adrenal gland shows a characteristic pattern over life, suggestive of functional roles, although few have been fully elucidated. In this Chapter, we will focus on the possible role of DHEA in learning and memory, either as the free steroid or the sulphate ester DHEAS.

Endocrine production of DHEAS begins with a high output from the fetal adrenal and in association with placental uptake, desulphation and further metabolism, is the major contributor of maternal estrogens during late pregnancy. However, adrenal DHEAS production declines after birth and plasma concentrations of DHEAS then remain low in humans until the age of 6-9 years, when they rise at the onset of adrenarche. Circulating DHEAS concentrations continue to rise during puberty, reaching a peak of around 5 μM during the second decade of life although there is variability between individuals and concentrations are generally higher in males than in females (Smith et al. 1975; Orentreich et al. 1984; Campbell 2006). A slow decline in the production of DHEAS then follows through adulthood, culminating in noticeably low plasma concentrations in the elderly (Orentreich et al. 1984).

With the above age-related changes in adrenal DHEAS production, there has long been an interest in the influence of DHEA on learning and memory. This was

reinforced nearly 30 years ago by the discovery that DHEAS and smaller amounts of the free steroid DHEA could be detected in adult male rat brain at concentrations which did not change upon removal of the adrenal glands and testes (Corpechot et al. 1981). This led to the concept of neurosteroids: steroids produced within the nervous system. In addition to rodents, the presence of DHEA and DHEAS has been reported in the brains of humans (Lacroix et al. 1987; Lanthier and Patwardhan 1986), other primates (Robel et al. 1987), amphibians (reviewed in Mensah-Nyagan et al. 2001) and avians (Migues et al. 2002; Tsutsui and Yamazaki 1995). Nevertheless and ironically in view of the fact that characterization of DHEA in rat brain led to the concept of neurosteroids, the synthesis of DHEA in the mammalian central nervous system remains a controversy. In endocrine glands, the CYP17 (17α-dehydrogenase/17,20-lyase) enzyme catalyses the production of DHEA from pregnenolone via the intermediate 17α-hydroxyprogesterone. This enzyme shows little or no activity in brain tissue (Mellon 2007) and adult male rat brain, which contains both pregnenolone and DHEA, lacks detectable concentrations of the above intermediate (Ebner et al. 2006). Thus, the possibility remains that as in other tissues, circulating DHEAS is imported into the brain and desulphated to DHEA. Consideration of the role of DHEA and DHEAS in learning and memory is further complicated by two factors: 1. that no specific receptor sites have been identified for either of these steroids in brain and 2. that apart from desulphation of DHEAS to DHEA, little is known at present of their downstream metabolism in brain to potential neuroactive steroids. In this Chapter, we will start by reviewing the possible sites of action for DHEA and DHEAS in the brain then outline the processes thought to underlie learning and memory before discussing how these might be influenced by DHEA(S).

2. Modes of Action of DHEA(S) in the brain

Steroid molecules classically bind to intracellular nuclear or cytoplasmic receptors to regulate gene expression. As mentioned above, DHEA appears to have no such receptor and so effects on transcription would appear to depend on metabolism to other steroids, which in peripheral tissues are known to include both androgens and estrogens. However, little is known of DHEA(S) metabolism by brain other than 17-keto reduction to produce free and sulphated androstenediol *in vivo* (Kishimoto and Hoshi 1972). Such metabolism could underlie the androgenic actions of DHEA in mouse brain, although some such activity can be detected even after blocking metabolism to the more potent androgens testosterone and dihydrotestosterone (see Mo et al. 2009). In addition, there are well-documented anti-glucocorticoid actions of DHEA, which occur also in the brain (see Maninger et al. 2009) and could involve interactions at the receptors for glucocorticoids and/or competition with the metabolic activation of these stress hormones (Muller et al. 2006). Whatever the mechanism, such actions of DHEA are likely to be of significance for consideration of the effects of stress on learning and memory, particularly with age.

As well as actions mediated probably indirectly through nuclear steroid receptors in the brain, DHEA(S) has been shown to have direct effects on neuronal membranes, especially at receptors for neurotransmitters. The latter include antagonism at type A receptors for the inhibitory amino acid transmitter γ-aminobutyric acid ($GABA_A$ receptors; Majewska et al. 1990; Spivak 1994) and enhancement of N-methyl-D-aspartate (NMDA) type receptors for the excitatory amino acid glutamate (Compagnone and Mellon 1998; Lhullier et al. 2004). DHEA(S) also shows agonism at type 1 sigma (σ_1) receptors (Urani et al. 1998)

which underlie further interactions at NMDA and acetylcholine receptors in the brain (Monnet et al. 1995; Matsuno et al. 1994; also see review by Monnet and Maurice 2006). Overall, the above actions on the neuronal membrane could be considered as excitatory. However, their relevance to any physiological influences of DHEA(S) on learning and memory must be approached with caution as such actions are reported at concentrations of these steroids higher than those observed in brain tissue *in vivo.*

Drugs such as barbiturates and benzodiazepines which are agonists and/or enhance the action of GABA at $GABA_A$ receptors are associated with impairment of memory acquisition Thus it might be expected that the $GABA_A$ antagonistic action of DHEA(S) would enhance learning (Chapouthier and Venault 2002; Castellano and McGaugh 1990). Likewise, the ability of DHEA(S) to enhance postsynaptic NMDA receptor function will increase the influx of Ca^{2+} ions, a form of signalling associated with the phenomenon of long-term potentiation of synaptic transmission and thought to underlie memory formation.

We will return to the above putative sites of action for DHEA(S) later in this Chapter but first give a brief overview of current concepts and experimental approaches to learning and memory.

3. Learning and Memory

Learning is typically understood as the process by which new information or knowledge is acquired, while memory is the process by which organisms store, retain and recall information. Memory formation is processed in three stages: acquisition (the initial phase in formation of a memory trace), consolidation (the phase during which the stabilisation of memory trace takes place) and retrieval (the "actualisation"

of the memory trace). Once formed, memory can last from minutes to years, suggesting the existence of complex mechanisms for altering patterns of neuronal connectivity involving changes in gene expression, protein synthesis and ultimately cellular structure.

Memory is often classified according to its endurance into short-term and long-term memory. Short-term memory has a limited capacity and allows recall for a period of several seconds to a minute without rehearsal. In contrast, long-term memory can store much larger quantities of information for a very long time, sometimes for a whole life span. However, research on different animal models and on humans indicates that long-term memory could be divided into at least two major types of memory systems: memory of "how" also called procedural (or implicit) memory, and memory of "that" also called declarative (or explicit) memory. Thus, we learn in two fundamentally different ways: we learn about the world around us acquiring knowledge of people and places and we learn how to do things, acquiring different skills. Procedural memory is not based on conscious recall of information in contrast to declarative memory, which requires conscious recall (Squire 1986; Squire 2004; Tulving 1985). According to information type carried, each one of these categories could be further subdivided into different subtypes, as shown in Fig 1.

(Insert Fig 1 here)

Why do we need model systems and different tasks to study memory? Because only by using different models and different tasks we might ultimately learn how different aspects of behaviour emerge from biological correlates of neural cell functions.

Standard laboratory tasks used in most memory laboratories today may be aversive or appetitive, single or multiple trial. They include passive avoidance and fear conditioning (both single trial) and versions of the Morris water maze to test spatial memory (multiple trials). All of them might be used to study short-term and long-term memory, declarative as well as non-declarative (procedural). The merit of one trial tasks is that they are sharply timed; the brevity of the training trial allows for a separation of events surrounding the training experience from the processes that occur during memory consolidation. However, during passive avoidance training and fear conditioning animals could acquire both declarative memories and procedural memories. Because procedural memories takes many more trials to acquire than declarative memories, measuring retention latencies after one-trial passive avoidance training most likely reflects declarative fear memories. Nevertheless, single trial learning is not typical of learning in general, because many instances of animal and human learning are based on the acquisition of experience in a number of repeated trials, involving processes such as generalisation, categorisation and discrimination.

Our laboratory has a long-standing experience in using the chick as a model system to study memory. The training task that we employ is one-trial passive avoidance task, in which chicks learn to avoid pecking at a small bead coated in the bitter, distasteful but non-toxic methylanthranilate (MeA). The task has the merits of being rapid and sharply timed (for a review see Rose 2000). Chicks that are trained to peck a bead typically coated with 100% MeA (strong training) show a disgust reaction (backing away, shaking their heads and wiping their bills) and will avoid a similar but dry bead for at least 48 hours. Another version of the task exists, where the bead is coated in 10% MeA (weak training) and chicks show avoidance for up to 8 h subsequently. Because the pecking response requires a positive, accurate act by the

bird, it also controls for effects on attentional, visual and motor processes. In its strong form the task can be used to identify the molecular cascade involved in memory formation and the interventions that impair consolidation; in its weak form the task can be used to explore potential memory enhancing agents.

Because of their closer evolutionary relatedness to humans, mammals are usually preferred to birds as model systems. However, the bird brain is not a primitive form of the mammalian brain, and has striking homologies and analogies with that of the mammal. Interestingly, birds have cognitive capacities that were thought to be the characteristic of primates. For example, sensory learning in songbirds shares important features with forms of sensory imprinting described in mammals and, similarly to mammals, results in a long-lasting and perhaps permanent memory (for review see Bolhuis and Gahrb 2004; Reiner et al. 2004).

The biological mechanisms that underlie learning, memory storage and eventually change in behaviour involve a cascade of molecular events such as intercellular communication, intercellular messenger systems, cell adhesion molecules, growth factors, and gene expression. In chicks, the combination of interventive and correlative studies has revealed a cascade of molecular processes occurring in defined brain regions, notably the left *intermediate medial mesopallium* (IMMP) and *medial striatum*. Briefly, within minutes of pecking at the bitter bead, there is: 1. enhanced glutamate release, 2. up-regulation of NMDA-sensitive glutamate receptors, and 3. the opening of N-type conotoxin-sensitive calcium channels. These transient synaptic responses result in the activation of protein kinases and the expression of immediate early genes such as c-fos and c-jun and subsequently, the family of late genes coding for glycoproteins which, inserted into the pre- and post-synaptic membranes, alter synaptic structure and connectivity. In

other words, the early phase of memory or short-term memory, depend on transient synaptic changes. Without activating transcription of new genes in the nucleus, short-term memories will quickly fade. The progression from short-term to long-term memory, a stable form of memory that can last from hours to life-time, requires the mobilisation of molecular processes that go beyond the synapse, to the neuronal nucleus, with activation of gene expression and protein synthesis to modify permanently synaptic structure.

4. Actions of DHEA(S) on Learning and Memory

4.1 Animal studies

The role of the steroid DHEA and its sulphate ester on learning and memory has been studied in animal models by using various learning paradigms. The first observations to suggest that DHEA and its sulphate ester may have memory enhancing properties in experimental animals were reported by Flood and colleagues. In a study using the T-maze footshock active avoidance test, immediate post-training intracerebroventricular (i.c.v.) injections of DHEA or DHEAS were found to facilitate memory retention one week later in adult male mice (Roberts et al. 1987; see Fig 2)). This improved retention was also seen after addition of DHEAS to the drinking water (Flood et al. 1988). A great deal of work has followed focusing on the physiological/pathological role of DHEA(S) in learning and memory processes and investigating their mechanisms of action. To examine their role during the acquisition, consolidation and retrieval stages of memory processing, these steroids have been administrated at various times throughout the learning process, as outlined below.

(Insert Fig 2 near here)

4.1.1 DHEA(S) pre-training

Subcutaneous (s.c.) administration of DHEAS to male mice 60 minutes before training on a passive avoidance task enhanced memory retention tested 24 hours later with a maximal effect at a dose of 1 mg/kg (Reddy and Kulkarni 1998a; Fig 3A).

(Insert Fig 3 here)

Additionally, DHEAS has been administered to both male and female adult rats trained on the elevated plus-maze test, where decreased transfer latency (the time taken by the rat to move into one of the enclosed arms) between training and 24 hours delay retention testing was used as a measure of learning and memory. DHEAS was shown to have a memory enhancing effect when administered 30 minutes prior to training at 5 mg/kg s.c., with testing done 24 hours later. Interestingly, the memory facilitating effects in this study were observed in male but not in female rats, demonstrating a sex-specific effect (Reddy and Kulkarni 1999). Another study has reported memory enhancement following DHEA administration (28 mg/kg, intraperitoneal (i.p.)) to male rats 30 minutes before training on the step-down inhibitory avoidance task, with testing carried out at both 1.5 hours and 24 hours later (Lhullier et al. 2004).

The memory enhancing effects of DHEA and DHEAS have also been examined in one-day old chicks using the one-trial passive avoidance-learning paradigm which, as mentioned above, has the advantage of a sharply-timed training event. Following direct injection 15 minutes before training into the IMMP, a brain region known to be specifically involved in the early stages of memory formation in these animals (see Rose 2000), both DHEA and DHEAS facilitated memory retention in male and female chicks tested 24 hours later. The DHEAS appeared more potent

than DHEA, with significant effects seen at minimum doses of 0.04 and 0.3 ng/chick, respectively. With the microinjection procedure employed, such doses would have resulted in concentrations at the site of injection of 22 μM for DHEAS and 208 μM for DHEA, before diffusion into the brain tissue (Migues et al. 2002). Also in chicks, a similar enhancement of memory retention tested 24 hours later was seen when DHEAS was administered at 20 mg/kg i.p. 30 minutes before training (Sujkovic et al. 2007; Fig 4A). Use of radioactively labeled DHEAS allowed an estimate of the amount of the i.p. dose reaching the brain and assuming a uniform distribution with no metabolism of the label, gave an estimated local concentration of around 0.5μM. The latter study also found the memory enhancing effect of DHEAS only to be significant in males and not females, as reported above for rats.

(Insert Fig 4 here)

4.1.2 DHEA(S) post-training

Several studies have examined the effects of DHEA and DHEAS on the consolidation of memory, by administering these steroids after training.

As already mentioned, an early study using the T-maze footshock active avoidance paradigm in adult male mice, showed immediate post-training i.c.v. injections of DHEA or DHEAS to facilitate memory retention one week later (Roberts et al. 1987; Flood et al. 1988). The same authors showed DHEAS also to enhance memory retention when administered i.c.v. at 30 and 60 minutes post-training, but not at 90 or 120 minutes. The most effective doses of DHEA and DHEAS would have resulted in local i.c.v. concentrations of around 20 mM, although these are likely quickly to have diluted on diffusion into the brain tissue. Enhancement of memory retention was also seen following systemic administration

of DHEAS to mice trained on the same task; the most effective doses being 20 mg/kg s.c. immediately after training or 43 mg/kg/day for one week in the drinking water (Flood et al. 1988). Other authors have shown DHEAS to facilitate memory retention for a passive avoidance task in male mice when injected s.c. 60 minutes post-training and tested 24 hours later, with the most effective dose being 1 mg/kg (Reddy and Kulkarni 1998a; Fig 3B).

In one-day old chicks trained on the one-trial passive avoidance memory task, post-training administration of DHEA (3 ng) or DHEAS (4 ng) into the IMMP could enhance memory retention when administered at 30 and 60 minutes post-training but not at 180 minutes (Migues et al. 2002). Similarly, i.p. administration of DHEAS 20mg/kg has been shown to enhance memory retention at 30 minutes and 4.5 hours post-training in male one-day old chicks trained on the above taste avoidance learning paradigm (Sujkovic et al. 2007; Fig 4B and C). In the latter study, as for DHEAS administered pre-training, effects were only significant in male and not in female chicks.

4.1.3 DHEA(S) pre-testing

Effects of DHEA and DHEAS on the recall of learning have been addressed by administering these steroids prior to recall of a learnt experience and have consistently failed to show significant effects on this aspect of memory function. Thus, DHEAS administered at 0.125 to 10 mg/kg, s.c. at 1 hour pre-retention test for the step-down passive avoidance task in mice had no effects on recall (Reddy and Kulkarni 1998a, Fig 3C). Likewise, Maurice et al. 1998 have reported that administration of DHEAS (20 mg/kg, s.c.) to mice 30 minutes prior to testing on the step-down type of passive avoidance task could not facilitate memory retention 14

days post-training. We have also been unable to detect changes in memory performance following administration of DHEAS 20 mg/kg i.p. 30 minutes before testing to one-day old chicks trained on the one-trial passive avoidance task (Sujkovic et al. 2007; Fig 4D).

4.1.4 Anti-amnesic effects

A number of studies have examined whether DHEA(S) could prevent and/or reverse pharmacological impairment of memory. Again, these show effects of DHEA(S) on acquisition and consolidation, rather than recall of memory.

Using the T-maze footshock active avoidance paradigm, Flood et al. (1988), reported anti-amnesic effects of DHEAS. In this study, 15 minutes prior to training, mice were administered with anisomycin (ANI) (20 mg/kg, s.c.), an inhibitor of protein synthesis, followed by immediate post-training injections of DHEAS (162 ng, i.c.v.) and an additional ANI injection at 1.75 hours later. Here, DHEAS was shown to reverse the amnesic effects of ANI when tested at one week post-training. It therefore appears that DHEAS may influence the process of protein synthesis that accompanies consolidation of memory. This is consistent with our observation that DHEAS enhances memory in the one-day old chick if administered at 30 minutes or 4.5 hours post-training (Sujkovic et al. 2007). These time points have been shown previously to be accompanied by protein synthesis which is crucial for the formation of memory in the chick model system (reviewed Rose 2000).

Using the same behavioural paradigm (Flood et al. 1988) have also studied whether DHEAS could reverse memory impairment induced by the muscarinic cholinergic receptor antagonist, scopolamine (SCO). Here, the learning deficit induced by SCO (1 mg/kg, s.c.) injected immediately after training could be reversed

by subsequent injection with DHEAS (162 ng, i.c.v.) at 45 minutes post-training. By contrast, DHEA (0.300, 1.350, 6.075 μg/kg) has failed to reverse a SCO (1 mg/kg) induced amnesia when administrated to mice trained on a spontaneous alternation task, a measure of short-term memory formation (Bazin et al. 2009).

Both DHEA and DHEAS could block ethanol induced memory impairment in male mice trained on win-shift foraging paradigm. In this study, steroids were injected at the dose of 0.05 mg/kg, i.p. 30 minutes prior to the testing trial, whilst ethanol was injected at 0.5 g/kg, i.p. at 10 minutes pre-testing (Melchior and Ritzmann 1996). Given that ethanol induced memory impairment can be potentiated by $GABA_A$ agonists such as muscimol but inhibited by its antagonists such as picrotoxin and bicuculline (Castellano and Pavone 1988), the observed effects of steroids in this study are consistent with their interactions with the $GABA_A$ receptor complex.

In another study, DHEAS at the doses of 5 and 10 mg/kg s.c. injected at 30 minutes prior to training could prevent the amnesic effects of the NMDA receptor antagonist dizocilpine (0.1 mg/kg, i.p.) administered at 15 minutes pre-training to mice trained on both the step-down passive avoidance and elevated plus maze tasks (Reddy and Kulkarni 1998b).

The possible interactions of DHEA and DHEAS with σ_1 receptors are further supported by two studies (Maurice et al., 1998; Reddy and Kulkarni 1998a) showing the memory enhancing effects of both these steroids given s.c. to be blocked in mice when co-administered with the σ_1 receptor antagonist haloperidol.

Indeed, the σ1 receptor may be a necessary target for anti-amnesic compounds because the reversal of dizocilpine-induced amnesia by PRE-084 (a selective σ1 receptor agonist) and by DHEAS in mice trained on spontaneous alternation and

passive avoidance task could be blocked by a 16-mer oligodeoxynucleotide antisense to the σ1 receptor cDNA which induced down-regulation of σ1 receptor expression in the brain (Maurice et al. 2001).

The above abilities of DHEA and DHEAS to reverse pharmacologically-induced amnesia are fully consistent with the sites of action for these steroids on neurotransmitter receptors described in Section 2.

4.1.5 Steroids and aging

Other animal studies have addressed the possibility that DHEAS can also ameliorate age-related impairments in learning and memory.

Flood and Roberts (1988), tested the effects of immediate post-training injection of DHEAS (20 mg/kg, s.c.) to middle-aged (18 months old) and old (24 months old) mice trained on the aversive T-maze footshock active avoidance task. Single treatment with DHEAS in this study was found to result in memory enhancement in both old and middle-aged animals when tested one week or one month after training, respectively.

Additionally, the effects of DHEAS have been examined on the impaired memory of 16 month as compared with 3 month old mice. When given before training on the step-down passive avoidance and elevated plus maze tasks, a dose of 10 mg/kg s.c. DHEAS significantly attenuated the age impairment of memory (Reddy and Kulkarni 1998b).

In another study, the effects of DHEAS on working memory as measured on the win-shift water escape task have been studied in 18-20 months old male and female mice. Here, post-training administration of DHEAS orally for 1 week, in the drinking water at the dose of 1.5 mg/mouse/day resulted in memory enhancement,

compared to mice given water alone, in both males and females (Markowski et al. 2001).

Using aged SAMP8 mice (a model to study amyloid beta toxicity, which is thought to be linked with Alzheimer's disease), one study has reported improved learning and memory when DHEAS was administered in drinking water (0.3 mg/mL) for 8 weeks to animals trained on the T-maze footshock avoidance paradigm (Farr et al. 2004).

4.1.5 Conclusions from animal studies

From the evidence of animal studies reviewed here, both DHEA and DHEAS are more likely to facilitate memory when administered pre-training and post-training but not pre-testing. Thus both these steroids appear to enhance the acquisition and consolidation but not the retrieval stages of memory. The sulphated steroid DHEAS is more potent in this respect than the free steroid DHEA, at least when a reliable comparison of their potencies can be made by direct injection into the chick brain (Migues et al. 2002). One possibility is that this higher potency of DHEAS arises at least in part from its slower clearance than DHEA from chick brain, as reported by Sujkovic et al. 2009 (see Fig 5). The latter study showed desulphation of a small proportion of the DHEAS injected into the brain to yield free DHEA during the period of memory formation and consolidation in the chick. Similar comparisons of the potencies of DHEAS and DHEA following i.c.v. injections in mice cannot be made as the studies reviewed here used different solvent vehicles and assessed the memory enhancing potencies of the free and sulphated steroids against different strengths of training. Nevertheless, several studies in rats and mice have shown inhibition of the Sts enzyme to increase the potency of DHEAS to enhance memory

and reverse drug-induced amnesia (see Johnson et al. 2000). Such effects should be interpreted with caution because these Sts inhibitors have been given peripherally, where they will inhibit desulphation of DHEAS in the liver (see Johnson et al. 2000) and are also sulphate esters themselves, competing with circulating DHEAS for access into the brain (Nicolas and Fry 2007).

(Insert Fig 5 here)

As for whether or not DHEA and DHEAS require further metabolism in order to induce memory enhancing effects, the evidence from reversal of pharmacologically-induced amnesia in animals is consistent with the actions of these native steroids at the neurotransmitter receptor sites identified *in vitro*. Also, our study of the metabolism of DHEA and DHEAS after direct injection into chick brain showed no detectable conversion to other steroids, at least during the time taken for the early stages of memory formation (Sujkovic et al. 2009). However, the animal studies described here have used intracerebral doses of DHEA(S) likely to produce localized concentrations of these steroids in at least the μM range and over 1000 fold higher than the endogenous brain concentrations (see Migues et al. 2002; Sujkovic et al. 2007; Ebner et al. 2006). Moreover, the other studies in which animals were administered DHEA(S) peripherally, frequently showed bell-shaped dose-response curves, suggesting an optimum dose beyond which additional actions become detrimental to learning and memory. More laboratory investigations are required into the actions and brain metabolism of DHEAS(S) at physiological concentrations. Inevitably, these will be limited by the low adrenal production of DHEA(S) in rodents and other laboratory animals in comparison to primates (see Cutler et al. 1978).

4.2 Human studies

Unsurprisingly, given the above evidence for the enhancement of learning and memory in animals by DHEA(S) and the well-known rise in the circulating concentrations of these steroids before puberty in humans, followed by a decline through adulthood, they have received attention not only as a markers for healthy cognitive development and ageing but also as potential treatments to enhance performance. Although some associations have been reported between plasma DHEAS and general mental health and performance in the elderly, there appear to be no significant correlations with more precise cognitive measures and DHEAS has no predictive value for future performance (see Wolf and Kirschbaum 1999; Vallée et al. 2001). Rigorous assessment of the clinical potential of DHEA(S) to enhance cognition, requires controlled investigations of DHEA supplementation of middle-aged or elderly subjects without dementia. A Cochrane Collaboration review of the literature to March 2008 could include only five such studies, with no evidence of a beneficial effect of DHEA supplementation on cognition (Grimley Evans et al. 2009). However, one study found that oral DHEA supplementation of elderly men and women for two weeks, sufficient to elevate their plasma DHEAS concentrations to young adult levels, protected against stress-induced deterioration in a test of attention (selecting target shapes on a sheet of paper; Wolf et al. 1998; see Fig 6). This is interesting in view of the anti-glucocorticoid effects of DHEA mentioned earlier, although the same study found DHEA supplementation to exacerbate the effects of stress on declarative memory (visual-verbal memory), possibly by enhancing cortisol release. Unlike DHEA(S), adrenal production of cortisol does not show marked age-related changes. This means that compared to healthy young adults with peak DHEA(S) production, children and the elderly have cortisol/DHEA(S) ratios 4-5

times higher in both blood and cerebrospinal fluid and so would be expected to be especially pre-disposed to the deleterious effects of stress on learning and memory (see Herbert 1998). Supplementation of elderly subjects for 3 months with DHEA sufficient to reduce the cortisol/DHEA ratio (as assessed in saliva) did indeed reduce confusion, anxiety and mood but had no significant effect on cognition, as measured in tests of speed, attention and episodic memory (van Niekerk et al. 2001).

(Insert Fig 6 here)

5. Concluding remarks

Studies in laboratory animals have consistently shown an enhancement of the acquisition and consolidation, rather than the recall, of memory by DHEA(S). Perhaps this gives some clue to the lack of clear effects in elderly human subjects, where recall of life skills and events might be expected to assume a greater importance. Nevertheless, controlled studies of learning and memory (of new information) in elderly human subjects have shown no significant effects of DHEA(S) supplementation. The ability of DHEA(S) to enhance the acquisition and consolidation of memory in laboratory animals is greater in males than in females. This may reflect actions mediated after conversion of the DHEA(S) to estrogens, which should already be higher in the females with functioning ovarian cycles. By the same logic and reverting to human subjects, DHEA(S) supplementation would be expected to have clearer effects in elderly, post-menopausal women than men. We suggest this because although both groups will have declining adrenal DHEA(S) production, men should still receive a contribution of these steroids from the testes (see Labrie 2010). Supplementation of healthy elderly subjects with DHEA was

indeed reported to improve mood in women rather than men (Wolf et al. 1997) but, as in other studies, there were no convincing improvements in cognitive performance.

So why have clear actions of DHEA(S) on learning and memory in laboratory animals not been followed by equally convincing studies in humans? There are probably several answers to this question including at least the following: 1. the age-related changes in DHEA(S) will vary between individuals 2. circulating DHEA(S) will fall more rapidly with age in women than men 3. supplementation may occur too late in the decline of DHEA(S) with ageing 4. supplementation is usually with oral DHEA which can elevate plasma DHEAS but is likely also to produce other steroid metabolites 5. the effects of DHEA(S) on cognition will vary according to the levels of the stress hormone cortisol and 5. there could be more than one mode of action for DHEA(S) and/or its metabolites.

In our view, more animal studies are required to elucidate the metabolism and mode of action of DHEA(S) in the brain. Such studies can be expected to inform clinical investigations of human subjects, which need to be sufficiently powered to uncover significant effects through the variables listed above.

Summary Points

- DHEA(S) enhance the acquisition and consolidation stages of memory in animal models of memory.
- DHEA(S) can reverse pharmacologically-induced amnesia in animal models.
- DHEA(S) does not appear to enhance recall in animal models of memory.
- DHEA(S) may play a role in age dependent cognitive decline.
- DHEA(S) supplementation has not yet been convincingly shown to enhance learning and memory in normal human ageing.

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FIGURES

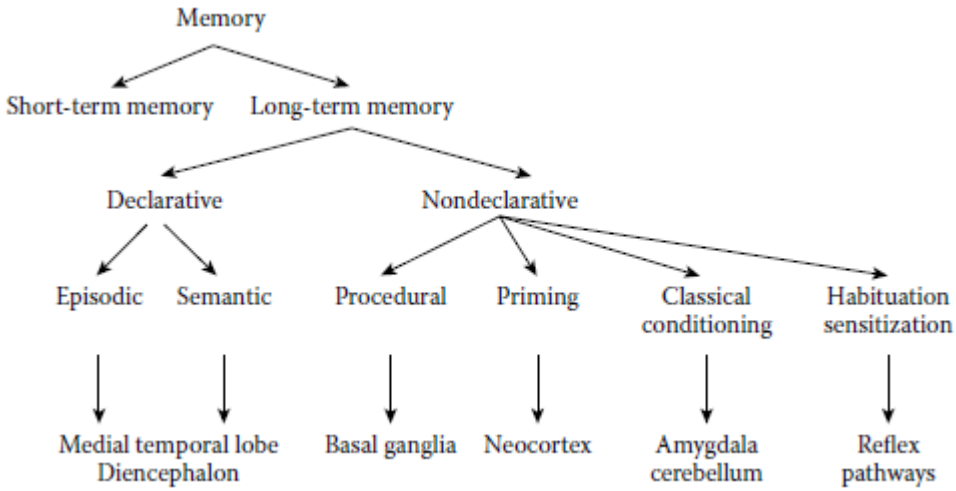

FIGURE 1 Taxonomy of memory: the multiplicity of memory systems and brain regions involved.

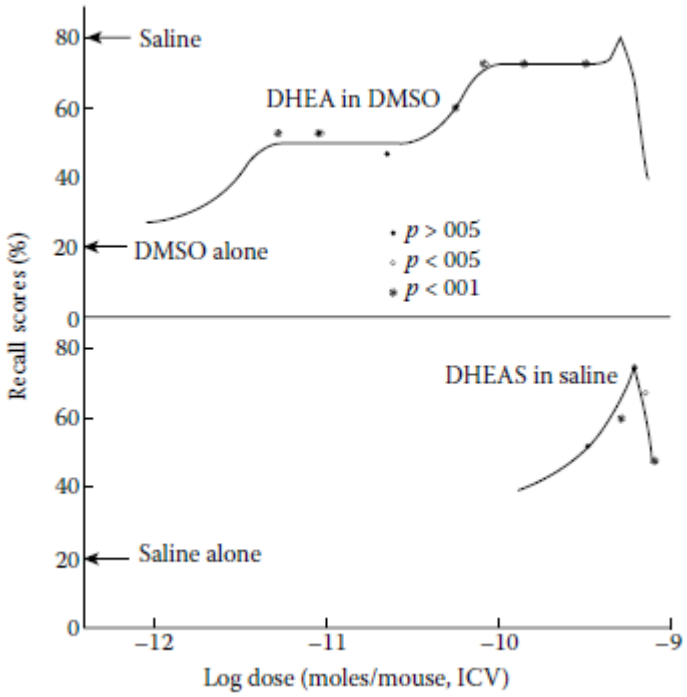

FIGURE 2 Effects of intracerebroventricularly injected DHEA and DHEAS on memory retention in mice. (a) DHEA and DMSO in well-trained animals: five

training trials in the T maze; the buzzer, loud; intertrial interval, 45 seconds; footshock level 0.35 mA. (b) DHEAS in saline in poorly trained animals; four training trials : buzzer, muffled; intertrial interval, 30 seconds; footshock level, 0.30 mA. Injections of test solutions were made within 3 minutes after training. Retention was tested 1 week after the training trials. (Reprinted from Roberts, E., L. Bologa, J.F. Flood, and G.E. Smith. 1987. *Brain Res* 406(1-2):357-62. Copyright (1987), with permission from Elsevier.)

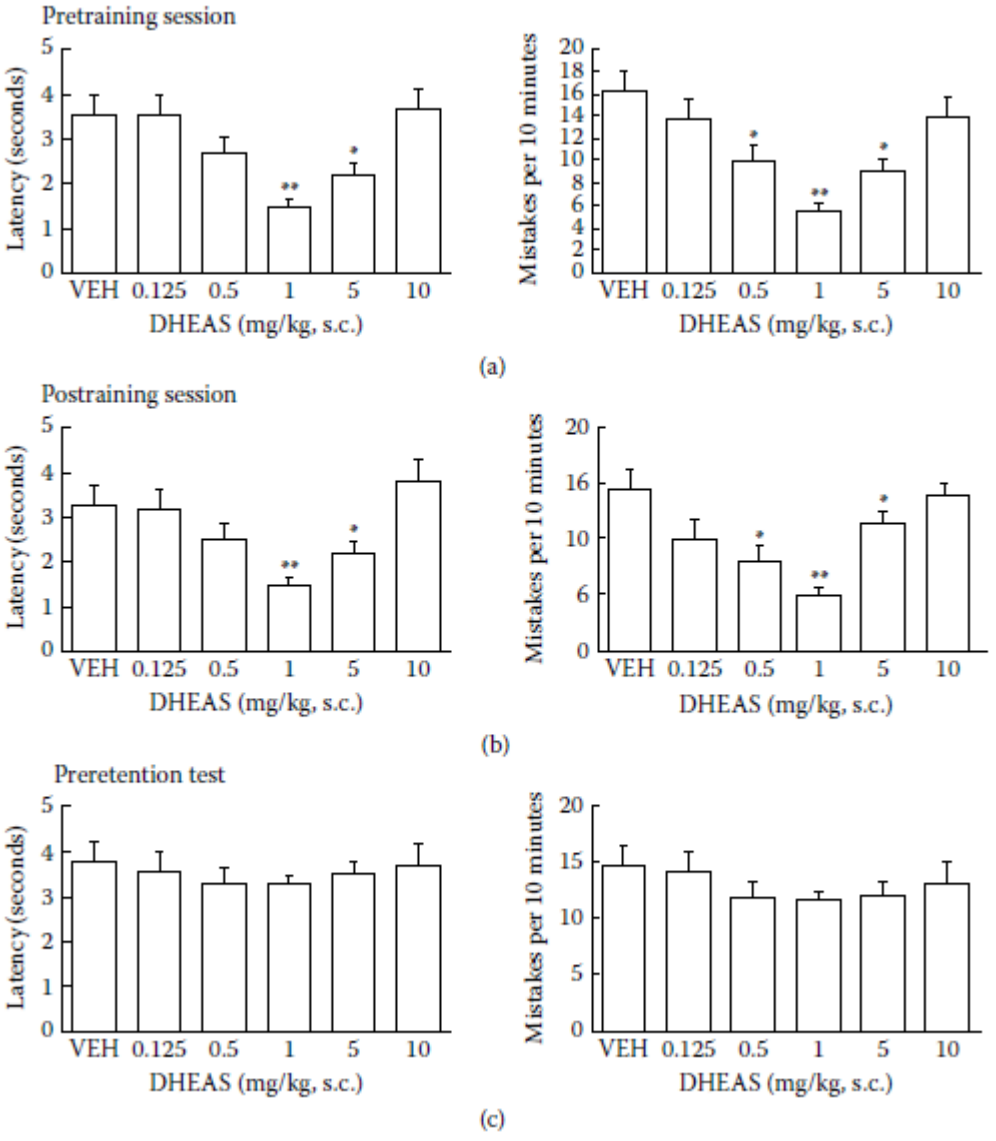

FIGURE 3 The effect of dehydroepiandrosterone sulphate (DHEAS); 0.125-10 mg/kg) administered subcutaneously to mice (a) 60 minutes before (pretraining), (b) immediately after (post-training) the training session or (c) 60 minutes before the retention test. The training was for passive avoidance of an electric shock to the feet, as tested by the step-down latency (left panel) and the number of mistakes (right panel). The retention test was performed 24 hours after the training session. The results are shown as mean ± standard error of the mean with six to eight animals per group. Values marked * or ** were significantly different from those obtained with mice injected with the solvent vehicle (VEH, 0.1% Tween 80 in saline) alone. (Reprinted from Reddy, D.S., and S.K. Kulkarni. 1998a. *Brain Res* 791(1-2):108-16. Copyright (1998), with permission from Elsevier.)

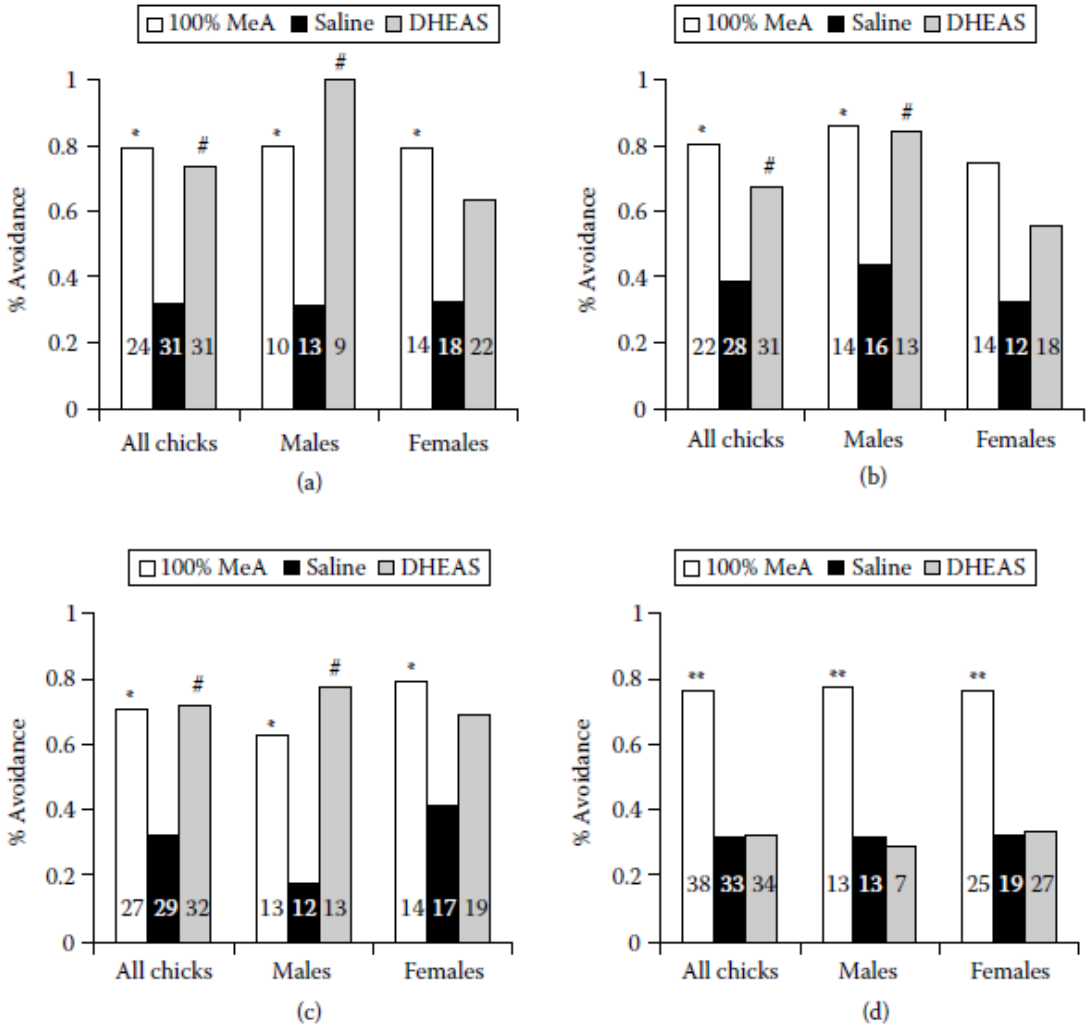

FIGURE 4 The effect of DHEAS (20 mg/kg) or saline administered i.p. either (a) 30 minutes before training, (b) post-training at 30 minutes, or (c) 4.5 hours or (d) 30 minutes before pretesting on recall for the weak aversive stimulus (WS) of 10% methylanthranilate (MeA). Non-injected controls were trained with the strong stimulus (SS) of 100% MeA. Recall was tested 24 hours after training and the results presented as percent avoidance. A statistically significant difference was observed when percent avoidance was compared between (*) SS group and saline injected WS chicks or between (*) DHEAS-injected WS group and saline

alone, also before WS. Numbers of animals in each group are presented in the relevant bars. (Reprinted from Sujkovic, E., R. Mileusnic, J.P. Fry and S.P. Rose. 2007. *Neuroscience* 148(2):375-84. Copyright (2007), with permission from Elsevier.)

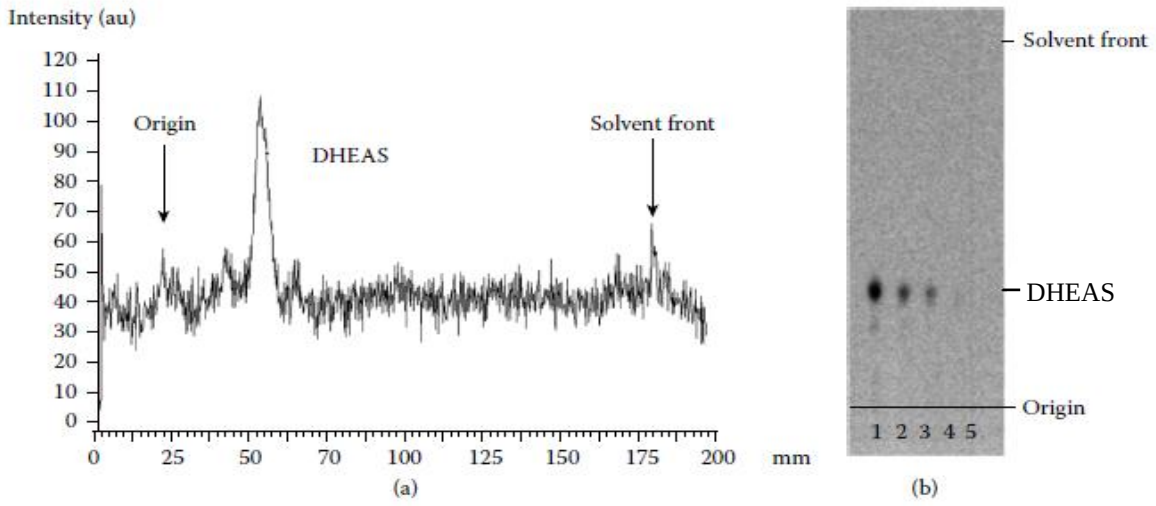

FIGURE 5 Typical chromatographic profile (a) from thin layer chromatography (TLC) of steroid sulphate fraction extracted from chick brain at 5 minutes after an intracranial injection of ^{3}H-dehydroepindrosterone sulphate (DHEAS) to the intermediate medial mesopallium and (b) phosphorimage of TLC for steroid sulphate fractions at: (1) 5 minutes, (2) 10 minutes, (3) 30 minutes, (4) 1 hour, and (5) 5 hours after injection of this label. The peak for DHEAS is labelled. (Reprinted from Sujkovic, E., R. Mileusnic, and J.P. Fry. 2009. *J Neurochem* 109(2):348-59. Copyright (2009), with permission from John Wiley and Sons.)

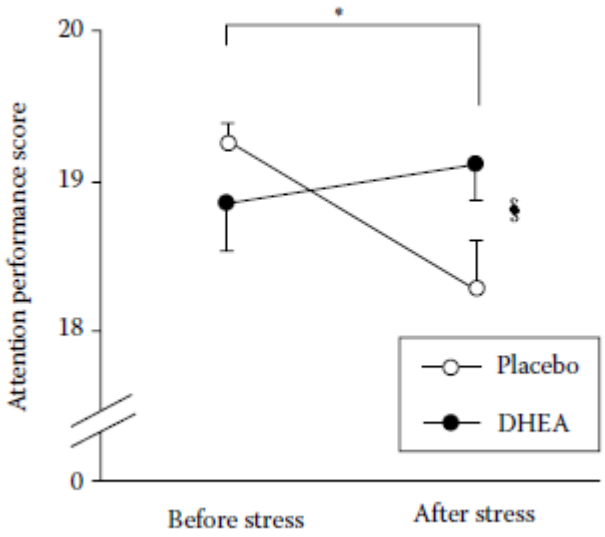

FIGURE 6 Effects of DHEA and stress on human performance in an attention test. Performance (using parallel versions) was tested before and after stress. The symbol * indicates a significant difference between pre- and post-stress performance for subjects given placebo and the symbol § a significant difference between DHEA and placebo. (Reprinted from Wolf, O.T., B.M. Kudielka, D.H. Hellhammer, J. Hellhammer, and C. Kirschbaum. 1998. *Psychoneuroendocrinology* 23(6):617-29. Copyright (1998), with permission from Elsevier.)