

**Testing cognitive performance of socially housed monkeys:
possibilities and limitations of the study of social influences on
age-related cognitive decline**



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2004

Front and back: Milva in the test apparatus (by Ido B. Toxopeus)
Inside photograph: A small part of one of the social groups housed at the Ethology Station (by Griet Nijs)
Cover design: Sandra van der Putten, Bite Grafische Vormgeving

ISBN: 90-393-3864-7

**Testing cognitive performance of socially housed monkeys:
possibilities and limitations of the study of social influences on
age-related cognitive decline**

Het testen van cognitieve prestatie van in groepen gehuisveste apen:
mogelijkheden en beperkingen bij het onderzoek naar sociale invloeden op
leeftijd-gerelateerde cognitieve achteruitgang

(met een samenvatting in het Nederlands)

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de
Rector Magnificus, Prof. dr. W. H. Gispen, ingevolge het besluit van het
College voor Promoties in het openbaar te verdedigen op

maandag 15 november 2004 des middags te 4:15 uur

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Financiële bijdragen werden verkregen van:
College van Bestuur van de Universiteit Utrecht
Lucie Burgers Stichting voor Vergelijkend Gedragsonderzoek, Arnhem

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CHAPTER 1

GENERAL INTRODUCTION

TOWARDS A MODEL OF AGE-RELATED COGNITIVE DECLINE IN SOCIALLY HOUSED MONKEYS

Long-term factors affecting age-related cognitive decline: the importance of social history

Human studies

Western societies are faced with an ageing population. Therefore, the study of the effects of ageing on cognitive capacities such as memory and learning receives much attention. As a group the older part of the healthy human population shows a relative deficit in a wide range of cognitive tasks, such as learning, in comparison to their younger counterparts (e.g. Bäckman et al., 2000). However, large individual variation in cognitive abilities exists. Whereas some individuals show strongly diminished cognitive capacities, others perform as well as their younger counterparts (Rowe & Kahn, 1987; 1997; Ylikoski et al., 1999). Furthermore, in a meta-analysis of studies on cognitive capacities and ageing Nelson & Dannefer (1992; see also Christensen et al., 1994) demonstrated an increase in variability in the reported results with increasing age of the subjects. This variation has prompted researchers to examine factors that may have a differentiating effect on the cognitive performance of old individuals.

A broad range of factors, from genes (Sole-Padulles et al., 2004) to environmental factors like pesticides (Bosma et al., 2000), have been suggested to influence age-related cognitive decline. Both cognitive performance at the first measurement, 'baseline cognition', and educational level are consistent predictors for subjects to maintain their level of performance at a second measurement, several years later (Arbuckle et al., 1986; Albert et al., 1995; Farmer et al., 1995). Similarly, occupational level has been shown to be related to the maintenance of performance in old age (Arbuckle et al., 1986; Schaie, 1990). The combined number of certain 'biological life events' that an individual experiences, such as surgical interventions under general anesthesia, was also reported to constitute a risk factor for cognitive functioning (Jolles et al., 1993). Yet, a more recent study failed to find a separate effect of the number of surgical interventions under general anesthesia on age-related cognitive decline (Dijkstra et al., 1998). Some of the previously reported factors have recently been questioned. For instance, recent work suggests that the factor of physically and 'intellectually engaging' activity (Albert et al., 1995; Hultsch et al., 1999) may not be independent from baseline cognition (Aartsen et al., 2002; Bosma et al., 2002). Besides activity, 'dietary style' is another aspect of the life style of people. Solfrizzi et al. (2003) have reviewed evidence regarding the effects of micro- and macro-nutrients on cognitive functioning, and concluded that dietary styles affect cognitive decline.

An important hypothesis concerns long-term exposure to elevated levels of glucocorticoids as a mediator of age-related cognitive decline (Lupien et al., 1994; Seeman et al., 1997; Lupien et al., 1998). Lupien and co-workers (1994) described that elderly people with rising basal levels of glucocorticoids over a period of five years showed a relatively accelerated cognitive decline.

In animal studies prolonged elevation of the levels of glucocorticoids is known to be related to social factors (e.g. Sapolsky, 1994; Blanchard et al., 2001). Yet, the possible risk factors associated with the social environment have, so far, received relatively little attention in human studies.

However, the finding that a low level of social support predicted a higher rate of cognitive decline in old age (Arbuckle et al., 1992; Seeman et al., 2001; Zunzunegui et al., 2003) points to the importance of including social factors in the study of age-related cognitive decline. Seeman and colleagues (2001) measured change in cognitive performance over a period of 7.5 years and reported that a "socially and emotionally supportive environment" (p. 250) protects against cognitive decline. They suggested that differential exposure to elevated levels of glucocorticoids played a mediating role in their finding. These glucocorticoids play an important role in the stress response.

Stress

Stress is most often considered to be the physiological response to uncertainty, insecurity, uncontrollability and threatening situations (Sapolsky et al., 1986; Lupien & Lepage, 2001). A release of glucocorticoids, such as cortisol, is an important aspect of the stress response that allows an individual to deal with the stressor (McEwen & Sapolsky, 1995). Although, the stress response has a clear immediate advantage to the individual, prolonged exposure to elevated levels of glucocorticoids has deleterious effects on health and cognition (Sapolsky et al., 1986; McEwen & Sapolsky, 1995; De Kloet et al., 1999; Belanoff et al., 2001). The hippocampus is known to be especially affected by prolonged elevated levels of glucocorticoids (McEwen & Sapolsky, 1995; Lupien & Lepage, 2001). For instance, Bodnoff et al. (1995) and Conrad and co-workers (1996) reported that cognitive impairments following prolonged stress in rats were related to reduced hippocampal functioning. Also, prolonged exposure to glucocorticoids has been related to impaired functioning of the prefrontal cortex (Lyons et al., 2000b; Mizoguchi et al., 2000).

Over a life time individuals may be differentially exposed to physical and psychological stressors (Sapolsky et al., 1986; Sapolsky, 1994). So, the increase in variation in cognitive performance with age could be explained by differences in exposure to elevated levels of glucocorticoids over the life span of individuals.

The social environment is a source of potent stressors in humans, non-human primates¹, and rodents (Sapolsky, 1994; Seeman & McEwen, 1996; Sapolsky et al., 1997; De Ridder, 2000; Blanchard et al., 2001; Abbott et al., 2003).

The studies of Seeman and co-workers (2001) and Lupien et al. (1994; 1998) provide preliminary support for the influence of prolonged social stress in humans. However, these papers covered only a small period (7.5 and 5 years, respectively) of the human life span. Thus, the effects were perhaps due to an acute effect of glucocorticoids (Porter et al., 2002), rather than to an accumulation of negative effects of elevated levels of glucocorticoids.

Research on depression in humans (Bremner et al., 2004), primates (Lyons et al., 1999), rodents (Krugers et al., 1997), and tree shrews (Fuchs & Flugge, 2002), has also shown the important effects of prolonged exposure to elevated levels of glucocorticoids and does relate longer periods of stress to cognitive impairments. Depression has been associated with a lack of social support, prolonged elevation of stress levels, structural change of the hippocampus, and cognitive decline (Van Londen et al., 1998; Belanoff et al., 2001; Sheline et al., 2003; Bremner et al., 2004). Still, the increased inter-individual variation mentioned above, concerns healthy individuals, rather than those affected by a disease such as depression.

Unfortunately, due to the complexity of the human social system and human longevity, the influence of long-term effects of the social environment is difficult to assess in humans. We propose that socially housed primates may provide a model to investigate these long-term effects of social position on cognitive performance.

The primate model

Primates are considered to be good models to study the processes involved in age-related cognitive decline. Primates show similar neuro-anatomical and performance changes with age as humans do (Price et al., 1994; Gallagher & Rapp, 1997; Voytko, 1997). Furthermore, like humans, primates show considerable variation in the extent of cognitive decline with age (Rapp & Amaral, 1992; Gallagher & Rapp, 1997; Voytko, 1997). Nevertheless, most studies have not used the full potential of this model.

Almost all studies on primate cognitive capacities have used animals that were housed solitary, at least for the duration of the experiment. By doing so these studies have neglected the influence that the natural social life exerts. There are two reasons why the value of the primate model for cognitive functioning can be increased by taking the natural social environment of primates into account.

First, the social environment is essential for a normal cognitive and emotional functioning in primates (Suomi, 1991a; Sanchez et al., 1998; Lyons et al., 1999; Sanchez et al., 2001; Lutz et al., 2003). Secondly, social housing creates

¹ Henceforth: "primates"

the possibility to study the long-term influence of the social environment on cognitive function.

There are several indications that life time dominance position in primates may be an important variable in explaining inter-individual variation in age-related cognitive decline.

Similar to what has been suggested in humans, prolonged exposure to elevated levels of glucocorticoids, in relation to the social environment, is a likely candidate to affect primate age-related cognitive decline. Findings comparable to those reported in humans (Seeman & Crimmins, 2001) point in that direction. Several authors reported that social subordination was related to health reduction in primates (Cohen et al., 1997; Shively et al., 1997; von Holst, 1998). Additionally, two observational studies reported a negative effect of a life time of social subordination on behavioural rigidity in old monkeys (Veenema et al., 1997; Veenema et al., 2001). Veenema and colleagues suggested that this indicated more pronounced cognitive decline in subordinate monkeys as compared to dominant animals.

The relation between dominance position and elevated basal levels of glucocorticoids in primates is complicated, and depends on the species-specific social system, on the stability of the social group and on housing conditions (meta-analyses: Abbott et al., 2003; Goymann & Wingfield, 2004). For instance, in unstable groups of baboons dominant males were shown to have relatively high levels of glucocorticoids when their position was challenged (reviewed in Sapolsky, 1994). In captive stable social groups of long-tailed macaques (*Macaca fascicularis*) low dominance status in females is often associated with an elevated levels of glucocorticoids (Welker et al., 1992; Shively et al., 1997; Shively, 1998; Abbott et al., 2003).

These findings are in line with the reports that subordinates are generally exposed to higher levels of psychosocial stress (for a review: Sapolsky, 1994). Subordinates generally receive high rates of unpredictable, redirected aggression from more dominant group members. They can exert little control over their environment, and have few outlets for frustrations, for instance after a lost conflict, because the number of animals to which aggression can be redirected is limited. On the other hand, the social group may have an important function in attenuating stress (Abbott et al., 2003), as has been reported in humans (Antonucci et al., 2000) and rats (Von Frijtag et al., 2000). For instance, when rats lost a conflict and were subsequently isolated they developed symptoms of depression, whereas rats that were socially housed after a lost fight did not (Von Frijtag et al., 2000). Nevertheless, subordinate primates usually have fewer social interactions than dominants (Melnick & Pearl, 1987; Shively et al., 1997; Shively, 1998). These results could be seen as analogues to the characteristics of little social support studied by Seeman and colleagues (2001).

Furthermore, dominance relations in many primate species constitute stable long-term factors in the lives of individuals. In long-tailed macaques (*Macaca fascicularis*) females remain in their natal groups while males migrate to other groups. All females of one family support each other against other families. Thus, the dominance rank of a female does not depend on her individual characteristics such as physical strength, but rather on the number and combined power of her female relatives. As a result females of one family are typically similar in rank as compared to the remainder of the group and the dominance position of females remains stable for many years (Walters & Seyfarth, 1987).

Thus, socially housed long-tailed macaque females, potentially, offer a valuable model to investigate the relation between long-term stress exposure and age-related cognitive decline, by taking life-time dominance history as an indication of life-time exposure to elevated levels of glucocorticoids. So, we hypothesise that a life time of subordination will accelerate age-related cognitive decline.

In an experimental assessment of cognitive capacity, the actual performance of an individual on a cognitive task will, clearly, not only be influenced by age and dominance history; it will also be influenced by more immediate factors. For instance, performance of both old and young subjects may be influenced by the motivation to engage in the task. Also, an investigation of cognitive capacity of socially housed monkeys in a cognitive test, will require temporary separation of subjects from their group, to avoid dominance relations affecting the choices in the task itself (Drea & Wallen, 1999). Moreover, social isolation may induce acute stress in subjects, which could affect their performance. Such 'immediate factors' may confound our ability to reveal the hypothesised effect. Therefore, to refine the proposed model we will also address the influence of 'immediate factors' on cognitive performance.

Immediate factors as determinants of cognitive performance independent of age

Human studies

In general, individuals perform well on a (cognitive) task when they are motivated to engage in it. Motivation is often operationalised in humans as the achievement goals of persons or the incentives given (Tomporowski & Tinsley, 1996; Lieberman, 1999; Hess et al., 2001; West & Yassuda, 2004). Motivation is hallmarked by excitement for the task at hand, leading to a state of general alertness, and concentration on the task (Mueller, 1992; Lieberman, 1999). Thayer (1996) has called this state 'energetic arousal'.

However, when an individual is anxious due to conditions related to the task he or she has to do, or due to other aspects of the surroundings, anxiety and acute stress will replace excitement. With such 'tense arousal' (Thayer, 1996)

concentration for the task and performance will decrease (Lupien et al., 1997; Newcomer et al., 1999; Vedhara et al., 2000; al'Absi et al., 2002). For example, a stressful public speaking task just prior to a test of cognitive capacity decreases performance (Lupien et al., 1997).

The two emotional states described above, of course, share similar expressions of arousal (see e.g. Lang, 1995). They may also interact with one another in the context of a cognitive test. For instance, motivation may be reduced by state anxiety (cf. Matthews et al., 2002).

Clearly, also temperamental differences influence cognitive performance of human subjects (Eysenck, 1985; Arbuckle et al., 1986; Revelle, 1987; Mueller, 1992; Evers et al., 1998; Kumari & Corr, 1998; Persson et al., 2000; Li et al., 2004). Differences in temperament reflect individual behaviour tendencies, which are relatively stable across time and situation and are related to differences in the expression of primary emotions (Goldsmith et al., 1987; Bates, 1989; Cloninger & Svrakic, 2000; Rothbart et al., 2000). Temperament comprises several aspects, which may also be referred to as 'traits'. For example, Persson et al. (2000) reported that persons with a high score on trait anxiety show reduced performance.

This general account provides a direction in which to address the performance and behaviour of socially housed monkeys in a cognitive test.

Primate studies

Since the 1970s an extensive body of literature has dealt with individual differences in behaviour in primates and other animals (for a review see Gosling, 2001). Most early studies have used subjective rating scales to assign scores on temperamental dimensions to individual subjects (e.g. Stevenson-Hinde & Zunz, 1978). Others used more objective scoring techniques to establish individual differences (e.g. Chamove et al., 1972; Van Hooff, 1973). Also, in later studies individual differences in temperament have mostly been studied by scoring observable behaviours, often in response to challenging situations (e.g. Suomi, 1987). Now, the importance of such individual differences in primates and many other animals, is widely recognised (Gosling, 2001). Nevertheless, only a few studies have looked at temperamental differences in relation to cognitive performance. Schneider and co-workers (1991) found that infant rhesus macaques (*Macaca mulatta*) with high scores on the temperament aspect trait anxiety (cf. Mueller, 1992; Persson et al., 2000) showed poor problem-solving performance. Additionally, cognitive performance of small-eared bushbabies (*Otolemur garnettii*) was positively related to individual differences in curiosity (Watson & Ward, 1996). Likewise, Stavisky and colleagues (2000) reported a positive correlation between 'sociality' and performance on a puzzle feeder task.

Our knowledge of the impact of acute stress and motivation on primate cognitive performance is also limited. Arnsten and Goldman-Rakic (1998) noted a negative effect of acute stress on cognitive performance of primates. Earlier,

Bunnell and colleagues (Bunnell et al., 1980; Bunnell & Perkins, 1980) had reported an effect of dominance status: high ranking male long-tailed macaques had poorer performance than lower ranking males. Possibly, the dominant animals experienced a higher level of acute stress than the subordinates did. Nash and Chamove (1981) reported that dominant animals were more 'disturbed' when isolated from the group than subordinates. Another possibility is that the dominant animals were less motivated to engage in the task than the subordinates were for other reasons. For instance, the drive to obtain food rewards may have been less in dominants. Obviously, these possibilities are not mutually exclusive; rather they are likely to be related to each other.

Long-term subordination and immediate factors as determinants of cognitive performance in socially housed monkeys

The dominance position of female long-tailed macaques may have an immediate effect on performance that is different from the long-term effect on cognitive ability. Over the course of a life time a subordinate position is expected to result in accelerated age-related cognitive decline, due to prolonged exposure to elevated levels of glucocorticoids. A previous study in our laboratory provided initial support for this hypothesis (Veenema, 1998), calling for additional work to confirm this relation between the rate of cognitive decline and social history of monkeys.

In contrast to the long-term effect, both old and young females with a dominant social position may perform poorly due to the 'concern' of maintaining a dominant position while isolated from the group. This latter effect may be mediated by differences in acute stress or motivation to engage in the task. Therefore, the possibility that the present dominance status of subjects will affect their acute stress and motivation to engage in the task is tested.

As previously stated, procedures to test socially housed monkeys will comprise elements, such as temporary isolation, likely to induce anxiety (i.e. acute stress) in subjects. Moreover, Higley and Suomi (1989) have reported that monkeys show individual differences in their reactions to social isolation. So, subjects with a high trait anxiety are likely to react to the procedures with a relatively high level of acute stress, and a low level of motivation to engage in the task.

Finally, high levels of acute stress are expected to be related to poor performance. A positive relation between performance and motivation to engage in the test is expected.

To recapitulate, an extended model is proposed to examine the psychosocial determinants of cognitive performance (Figure 1.1). The following hypotheses will be tested:

- 1) Lifetime subordination accelerates age-related cognitive decline.
- 2) High levels of acute stress are negatively related to performance.
- 3) High levels of motivation to engage in the task are positively related to performance.
- 4) High dominance is negatively related to motivation to engage in the task and positively related to acute stress, thus to reduced performance.
- 5) High trait anxiety is negatively related to motivation to engage in the task and performance, and positively to acute stress.

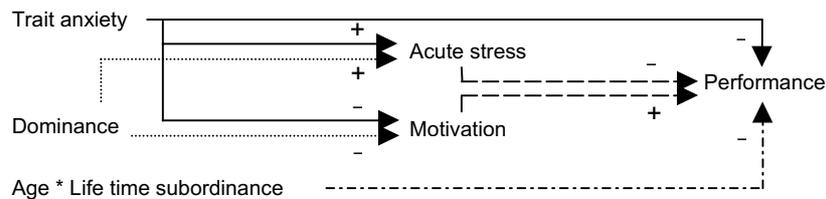


Figure 1.1. Schematic representation of the proposed model. Arrows are printed in different styles for reasons of clarity.

Stimulus features as determinants of cognitive performance

Up to this point, we focussed on factors relating to the subjects and their social position as possible sources of variation in cognitive performance. Next, we concentrate on the impact that features of the stimuli used in cognitive tests may have on cognitive performance.

Primates are important test-subjects in the field of animal learning and cognition. Discrimination and Discrimination-Reversal learning tests (see also the Subjects and methods section) have often been used in such studies. Many students of primate discrimination learning have used objects in their tests, but two-dimensional stimuli (pictures) have been used as well. The use of pictures has clear advantages over objects. Pictures can be automatically displayed on touch sensitive screens. This allows a high degree of control in experimental procedures, without the presence of an experimenter, who may induce anxiety and give inadvertent cues, to operate the presentation of stimuli. For instance, the inter-trial interval can be precisely set and maintained. Also, easy creation and manipulation of stimuli is possible, as well as control over the inter-, and intra-experimental constancy of stimuli (Fagot et al., 2000). Additionally, many more trials can, potentially, be presented to a subject, compared to when an experi-

menter is needed to present object-based stimuli. Furthermore, recent methods have used automatic identification of subjects to further automate testing procedures (Andrews, 1994). So, considering the advantages technology offers (e.g. Bhatt & Wright, 1992; Washburn et al., 1994; Dias et al., 1996b; Crofts et al., 1999), it is likely that the use of pictures in discrimination learning test will become predominant in the future.

Traditionally, stimuli used in visual discrimination studies were 'neutral' stimuli such as patterns or geometrical shapes (Moss et al., 1988; review: Anderson, 1998). Other authors have used stimuli depicting various objects. For example, Oveman and colleagues (1990) showed their subjects pictures of common household objects such as car keys, shoes and a screwdriver. Clearly, the stimuli used in the various studies differ in a number of aspects such as colour, shape, contrast, familiarity, and biological relevance. Although the choice of stimuli is obviously an important aspect of the design of a discrimination test, only scarce information exists concerning the possible effects of these differences on discrimination performance.

Several papers, mostly from the 1940s and 1950s, have reported the importance of colour in comparison to shape or in comparison to black-and-white stimuli (e.g. Harlow, 1945; see also Fobes & King, 1982). Yet, little attention has been paid to other features of pictorial stimuli. Nevertheless, several lines of direct and indirect evidence suggest that biological relevance may be one feature that needs to be considered. Primates have been shown to recognise objects depicted in pictures (reviews: Bovet & Vauclair, 2000; Fagot et al., 2000). Additionally, social stimuli (i.e. pictures of conspecifics) are preferred as rewards in cognitive tests (for a review see Anderson, 1998). These results raise the question whether subjects may learn a discrimination faster when they can recognise the objects depicted in pictorial stimuli compared to abstract stimuli.

Previously, some work was done in this respect. Rosenfeld and Van Hoesen (1979) and Dittrich (1988; 1994) have compared discrimination performance for biologically relevant, social stimuli with performance on tests with abstract stimuli. However, no clear conclusions can be drawn from these papers (see also Anderson, 1998). So, the question formulated above cannot yet be answered. We hypothesise that stimuli depicting biologically relevant objects improve performance relative to abstract pictures. An additional hypothesis was formulated to further examine the influence of stimulus features on discrimination performance. This hypothesis states that biologically relevant familiar stimuli are more easily discriminated than irrelevant familiar stimuli. Some familiar objects were biologically relevant: the animals had specific positive (or negative) previous associations with it. Other familiar objects were irrelevant: the animals had neutral previous associations with it. This difference in biological relevance of familiar stimuli may influence discrimination performance (cf. Leonards et al., 2002).

To recapitulate, the following two hypotheses concerning the influence of stimulus features on cognitive performance will be tested:

- 1) Stimuli depicting biologically relevant objects are more easily discriminated than abstract stimuli.
- 2) Stimuli depicting biologically relevant familiar objects are more easily discriminated than stimuli depicting irrelevant familiar stimuli.

SUBJECTS AND METHODS

Subjects

In this thesis we argue that socially housed monkeys are the most valid model of age-related cognitive decline in terms of face and construct validity. Data are derived from individuals with a more natural emotional and cognitive development than the commonly used solitary housed animals. Furthermore, social housing allows examination of the long-term influence of the social environment on age-related cognitive decline.

The colony of long-tailed macaques (*Macaca fascicularis*) housed at the Ethology Station of Utrecht University provides a unique opportunity to investigate the relation between dominance history and cognitive decline. The social groups housed at the Ethology Station are kept in a way that mimics the natural group dynamics. The groups are also similar to wild populations with respect to behaviour, group composition and dominance structure. Furthermore, the reported relation between social subordination and elevated glucocorticoid levels (Abbott et al., 2003) has also been found in this colony (Das, 1998). Furthermore, all animals are individually known and dominance relations in groups have been monitored throughout decades of ongoing studies. This allows us to classify subjects in terms of their global dominance history: high, middle or low ranking.

Procedures

To assess the influence of the social environment on cognitive functioning requires on the one hand social groups, and the other hand individual tests of cognitive performance. The relation between these two requirements is obviously strained, because subjects need to be temporarily isolated from their social group to avoid dominance relations affecting the choices in the test itself (Drea & Wallen, 1999). This temporary isolation should involve a minimum of separation stress. To this end groups were subjected to an extensive training programme. Animals were prompted to enter an enclosure next to their home cage from which they could only return to the preferred home cage through a 'learning compartment'. All animals of the groups were trained to do this voluntarily. Additionally, experimental subjects were gradually habituated to remain isolated in the learning compartment for the duration of a test session.

Discrimination-Reversal test

To test the proposed hypothesis that a life time of social subordination has an additional negative effect on age-related cognitive decline, a test must be used that will reveal the age-related cognitive decline and the detrimental effects of prolonged elevated levels of glucocorticoids. The Discrimination-Reversal (DR) test meets these demands. It has been widely used in primate cognition studies (e.g. Bartus et al., 1979; Dean & Bartus, 1988; Itoh et al., 2001), and it has also revealed the inter-individual variation between the old subjects (Anderson et al., 1996; Veenema, 1998; Voytko, 1999; Itoh et al., 2001) consistent with other studies (for a review: Gallagher & Rapp, 1997). Although the DR-test is recognised as a tool to study age-related cognitive decline (Dean & Bartus, 1988, Itoh et al., 2001), three papers have not found an age-related deficit in performance (Rapp, 1990; Lai et al., 1995; Anderson et al., 1996). However, the authors speculate that this could be due to large inter-individual variation (Anderson et al., 1996), or extensive previous experimental experience (Rapp, 1990), or the use of objects rather than pictures (Lai et al., 1995).

A DR-test consists of two phases. In the first phase subjects must learn which of the simultaneously presented stimuli is associated with a reward. This initial, simple discrimination is followed by the second phase, in which the reward is associated with the other stimulus. Subjects must now change their response pattern and stop responding to the previously rewarded stimulus in favour of the stimulus that is now rewarded. When subjects have also reached a predetermined level of correct choices on this reversal phase, one may reverse the reward association again. The DR-test is suitable to test the hypothesis because it may reveal the negative effects of prolonged elevated levels of glucocorticoids. It has been recognised as an important tool to assess the functioning of the prefrontal cortex (Lai et al., 1995; Roberts, 1996). The prefrontal cortex is considered important for associating stimuli with rewards and inhibitory control (Robbins, 1996). An impairment in inhibitory control will result in many wrong choices for the previously rewarded stimulus after a reversal (Lai et al., 1995; Dias et al., 1997; Lyons et al., 2000b).

Furthermore, the hippocampus also plays a role in performance on a DR-test. Even though the DR-test may be completed, using simple, hippocampus independent, associative learning, the hippocampus can play an important role in learning the rule behind the test (i.e. win-stay-lose-shift). The hippocampus is known to be important in the flexible use of learned information ('declarative memory' Eichenbaum, 1994, 2001). So, the integrity of the hippocampal system is important for cognitive flexibility, i.e. to switch response strategies (Rapp et al., 1996; Gallagher & Rapp, 1997; Voytko, 1999). This may result in a better performance. Subjects may need fewer trials to switch their response pattern, compared to associative learning.

So, performance on the DR-test is sensitive to a reduced functioning of the hippocampal system and the prefrontal cortex. A limited functioning of these

brain areas has been associated with prolonged exposure to elevated levels of corticosteroids (Sapolsky et al., 1986; Squire, 1992; De Kloet et al., 1999; Lyons et al., 2000b; Fuchs et al., 2001; Wellman, 2001).

SUMMARY

This thesis addresses the possibilities and limitations of testing cognitive performance of socially housed primates. Possibilities lie in the study of the relation between the social environment and cognitive functioning. In **Chapter 2** we will test the hypothesis that a life of social subordination accelerates age-related cognitive impairment in monkeys. A procedure will be used that allows socially housed monkeys to be individually tested in a Discrimination-Reversal (DR) test on a voluntary basis.

Limitations may be encountered in the responses of subjects to the testing procedures. In **Chapter 3** we will test whether the acute stress and motivation to attend to the task of subjects is related to performance on the one hand and present dominance status on the other. These predictions will be tested in relation to the cognitive performance of our subjects in the DR-test. Measures for acute stress and motivation will be extracted from the spontaneous behaviour of the subjects during the DR-test. This allows an assessment of these factors without interfering with the DR-test procedures.

The proposed model is extended further in **Chapter 4**. Individual differences in trait anxiety are predicted to influence both behaviour of subjects during the DR-test, as well as to have a direct detrimental effect on performance. Differences in trait anxiety will be assessed in a separate experiment in which subjects are exposed to an aversive acoustic stimulus, within the context of the social group. The behavioural reaction of the subjects will be analysed to provide a measure of trait anxiety. Thus, the Chapters 2, 3, and 4 can be seen as an attempt to identify the risk factors for cognitive performance in socially housed monkeys.

In Chapters 5 and 6 the focus is shifted from factors relating to the subjects to the test itself. Considering the advantages of modern technology using computer operated tests, and displaying two-dimensional stimuli on (touch sensitive) screens it is important to know whether, and how features of such stimuli influence performance.

In **Chapter 5** the hypothesis that stimuli depicting biologically relevant objects improve performance compared to abstract stimuli will be tested. The previously introduced DR-test will incorporate both abstract and relevant stimuli. A detailed analysis of the choices of the subjects will address the impact these types of stimuli have on their performance.

The effect of stimulus parameters on cognitive performance will be more closely examined in **Chapter 6**. An additional hypothesis is formulated concerning the possible difference between relevant and irrelevant familiar stimuli. To

test these hypotheses a series of two-choice discrimination tests will be used in which stimulus features are systematically varied.

In the final chapter we will provide a summary and integrated discussion of the findings. We will evaluate the possibilities and limitations of testing cognitive performance of socially housed monkeys.

CHAPTER 2

The influence of dominance history on
age-related cognitive decline in socially housed
long-tailed macaques (*Macaca fascicularis*)

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ABSTRACT

The extent of age-related cognitive decline varies considerably within the aged population of both human and primates. Unlike in humans, no information on what causes individuals to vary is available for primates. This paper reports a pioneering experiment examining the effect of the social environment on the age-related cognitive decline in group living female long-tailed macaques (*Macaca fascicularis*). A life time history of social subordination is taken to indicate chronic psychosocial stress exposure of the subjects. Aged female subjects with different dominance histories were compared with younger group members with regard to performance on a Discrimination-Reversal learning test. No additional negative effect of a life time of subordination on age-related cognitive decline was found. Methodological limitations and possible confounding variables are discussed.

INTRODUCTION

The cognitive capacities of both humans and primates slowly decline with age, although large inter-individual variation has been reported in both groups (Rowe & Kahn, 1987; Nelson & Dannefer, 1992; Rapp & Amaral, 1992; Price et al., 1994; Peters et al., 1996; Rowe & Kahn, 1997; Ylikoski et al., 1999). In human research several factors appear to be related to this variation. For instance, education and occupational level have been related to the maintenance of performance in old age (Arbuckle et al., 1986; Schaie, 1990; Albert et al., 1995). Also, Jolles and co-workers (Jolles et al., 1993) reported that the number of certain 'biological life events' increase the chance of cognitive impairment at later age. Moreover, certain life-style and personality aspects as well as a lack of social support appear to affect cognitive impairment (Winocur et al., 1987; Moscovitch & Winocur, 1995; Evers et al., 1998; Kumari & Corr, 1998; Persson et al., 2000; Seeman et al., 2001; Solfrizzi et al., 2003; Zunzunegui et al., 2003). The importance of these last two factors may be explained by the finding that extended exposure to elevated levels of glucocorticoids, due to psychosocial stress, also contributes to this inter-individual variation in humans (Lupien et al., 1994; Lupien et al., 1998). The current study is the first to explore one of the variables that may be of influence on the inter-individual variation in primates. We will focus on the possible influence of long-term social subordination on age-related cognitive decline.

Stress, life time dominance history, and age-related cognitive decline

One long-term factor affecting socially housed primates throughout their lives is their dominance position. This study used female long-tailed macaques (*Macaca fascicularis*) because of the stable dominance hierarchy between female group members. Groups of long-tailed macaques are structured along matriline, with females remaining in their natal groups and males migrating to other groups. The females of one family are all similar in rank compared to the remainder of the group and support each other against other families. Thus, the power and size of her family determines the rank of a female. Because of this group structure the dominance hierarchy of females remains stable for many years (Walters & Seyfarth, 1987). The dominance position of a female thus constitutes a stable long-term factor that could differentiate the cognitive abilities of old females via prolonged exposure to elevated glucocorticoid levels. The relation between dominance position and psychosocial stress is complicated, depending on the species specific social system, on the stability of the social group, and housing conditions (Abbott et al., 2003). In captive, stable social groups of long-tailed macaques low dominance status in females has been shown to be related to an increased exposure to elevated levels of glucocorticoids (Welker et al., 1992; Shively et al., 1997). Additionally, this relation was confirmed in an unpublished study in our laboratory (Das, 1998). Similar findings have also been reported for the closely related rhesus monkey (*Macaca mulatta*) (Gust et al., 1993). Further-

more, a recent meta-analysis by Abbot and co-workers (Abbott et al., 2003) provides additional support for this notion. Evidence suggests, therefore, that it is justified to use the life time history of the dominance status of a female as an indication of long-term psychosocial stress experienced by that female.

Although limited exposure to glucocorticoids has a beneficial effect on performance on any task, it is clear that prolonged exposure to elevated levels of glucocorticoids does limit the functioning of brain areas that are important in cognitive performance such as the prefrontal cortex (Lyons et al., 2000b; Mizoguchi et al., 2000; Wellman, 2001) and the hippocampus (Sapolsky et al., 1990; Squire, 1992; De Kloet et al., 1999; Fuchs et al., 2001).

Both these brain areas are known to play a major role in performance on cognitive tasks such as the Discrimination-Reversal test (Eichenbaum et al., 1992; Squire, 1992; Dias et al., 1997; Lyons et al., 2000b; Eichenbaum, 2001). Thus, the life time dominance history may have a differentiating effect on the age-related cognitive decline of female monkeys.

Seeman and colleagues (2001) have recently argued along the same lines. They suggested that their finding of an association between a less "socially and emotionally supportive environment" (p. 250) and poor cognitive performance was mediated by high "levels of physiological arousal" (p. 250). Furthermore, a first indication for the validity of this reasoning comes from two behavioural studies (Veenema et al., 1997; Veenema et al., 2001) showing that on average low-ranking old animals had more difficulty than high-ranking old group members in processing information from their social environment.

We hypothesise that aged females with a history of low rank show a cognitive impairment relative to aged females with a history of high rank.

METHODS

In a pilot study, prior to the present work, Veenema (1998) found that families of females with a low dominance status had a stronger decline in cognitive performance with increasing age than families with high rank. However, some methodological difficulties were encountered in this pilot study. For instance, with the level of the learning criterion used in combination with the chance level (50%) for correct choices one cannot exclude that subjects attained this learning criterion by chance. Furthermore, tests with a high chance level for correct choices may result in some females adopting a random response strategy. In this study we avoided these limitations, and we improved the method for studying the functioning of the prefrontal cortex by examining the perseveration of our subjects. This resulted in the methods described below; Table 2.1 summarises the methodological differences between the pilot and this study.

Table 2.1. Methodological differences between the pilot and current study

| | Pilot study | Current study |
|---|-----------------------------|------------------|
| Number of stimuli on each trial | 2 | 4 |
| Chance level for a correct response on any single trial | 0.5 | 0.25 |
| Number of reversals after each discrimination | 1 | 4 |
| Number of discriminations with a new set of stimuli | >1 | 1 |
| Single male used | Yes | No |
| Number of subjects | 13 | 15 |
| Number of families of females | 4 | 8 |
| Number of subjects in each family | 3 | 1-3 |
| For each family one female in every age category | Yes | No |
| Number of rank categories | 4 | 3 |
| Number of age categories | 3 | 3 |
| Statistics | Multiple Regr. ¹ | GLM ² |

¹ Multiple Regression

² General Linear Model

Subjects and Housing

Two groups of socially housed long-tailed macaques (*Macaca fascicularis*) participated in this study, the R-, and the L-group. Both groups were housed in large indoor (80 m²) - outdoor (200 m²) enclosures at the Ethology Station of Utrecht University. Both groups consisted of a number of adult males and females and their offspring. The total number of individuals in each group was 46 and 29, respectively at the start of testing. Fifteen female subjects were selected, nine from the R-group and six from the L-group. Because the number of old females was limited, six selected females from the R-group had previously participated in the pilot study four years earlier (for additional information on the subjects see Table 2.2).

In order to examine the variation in performance of subjects of different age classes in the two studies we defined three age categories: young adults aged between 5 and 9.5 years (category 1); middle aged between 9.5 to 14 years (2); old females aged 14 years or older (3). These categories were based on the information, provided by Tigges and colleagues (1988) and Moss (1993), that rhesus monkeys (*Macaca mulatta*) of 16 years of age can be considered to be old and comparable to humans of 80 years of age. Because rhesus monkeys are somewhat larger and have a larger brain size than long-tailed macaques (Rowe, 1996) the life expectancy of rhesus monkeys is probably also somewhat longer than that of long-tailed macaques (Hakeem et al., 1996). Therefore, it seems appropriate to call females of more than 14 years aged. As female long-tailed macaques reach sexual maturity between 4 and 5 years of age (Rowe, 1996) we set the lower boundary of the young age group at 5 years.

Table 2.2. Group, name, family, dominance rank, age, experience and performance data for the subjects

| Group | No. | Name | Family | D-H ¹ | Age ² | Age cat ³ | Exp ⁴ | Discrimination | | Reversals | |
|-------|-----|----------|--------|------------------|------------------|----------------------|------------------|----------------------------|----------------------------|--------------------------|--------------------------|
| | | | | | | | | Nr. of Trials ⁵ | Nr. of trials ⁵ | Persv. err. ⁶ | Persv. err. ⁶ |
| R | 1 | Felix | 1 | 1 | 27 | 3 | Yes | 1.91 | 2.43 | 52.00 | |
| R | 2 | Alfa | 1 | 1 | 12.8 | 2 | Yes | 2.07 | 2.44 | 45.80 | |
| R | 3 | Kraa | 1 | 1 | 9.3 | 1 | Yes | 1.98 | 2.14 | 57.81 | |
| R | 4 | Milva | 2 | 2 | 10.4 | 2 | No | 2.14 | 2.46 | 66.84 | |
| R | 5 | Yudea | 3 | 2 | 13.6 | 2 | Yes | 1.89 | 2.15 | 46.56 | |
| R | 6 | Freya | 3 | 2 | 9.9 | 2 | Yes | 1.82 | 1.87 | 41.72 | |
| R | 7 | Rastafa | 3 | 2 | 5.1 | 1 | No | 1.86 | 2.39 | 61.12 | |
| R | 8 | Roza | 4 | 3 | 18.0 | 3 | No | 1.54 | 2.53 | 49.43 | |
| R | 9 | Hoeba | 5 | 3 | 11.3 | 2 | Yes | 1.40 | 2.24 | 44.22 | |
| L | 10 | Lixa | 6 | 1 | 25.1 | 3 | No | 1.91 | 2.69 | 52.73 | |
| L | 11 | Ikea | 6 | 1 | 13.6 | 2 | No | 2.42 | 2.40 | 62.02 | |
| L | 12 | Virginia | 6 | 1 | 7.0 | 1 | No | 2.11 | 2.43 | 60.71 | |
| L | 13 | Bilboa | 7 | 3 | 9.1 | 1 | No | 2.06 | 2.53 | 56.01 | |
| L | 14 | Kaa | 8 | 2 | 16.1 | 3 | No | 1.86 | 2.55 | 63.71 | |
| L | 15 | Yukka | 8 | 2 | 7.0 | 1 | No | 2.38 | 7 | 7 | |

¹ Dominance history (see text for details)

² Age in years

³ Age category (see text for details)

⁴ Indicates whether subject had previous experience

⁵ LOG of number of trials to criterion

⁶ Mean percentage of perseverative errors

⁷ Data for this subject omitted from the analysis

All animals of both groups were individually known and the dominance relations have been monitored throughout decades of ongoing studies. This information on the dominance history of all group members allowed us to assign the subjects of this study to one of three broad rank categories. These rank categories (Table 2.2) signify whether females had a history of a high dominance rank (1), a medium dominance rank (2) or a low dominance rank (3) in their group. In the L-group a rare rank reversal occurred thirteen months before the start of the test, which affected the rank of three subjects. The dominance history is based on the situation prior to this rank reversal.

Experimental set-up and testing procedure

Testing was conducted in an experimental wire mesh cage (approximately 17 m², see Figure 2.1) situated in a room adjacent to the animals' home cage. The experimental cage was elevated 0.80 metres above the floor. A removable partition (Figure 2.1, c) separated the experimental cage from the home cage.

Inside the test cage, three separate compartments, A, B, and C, could be created by closing sliding doors 'a' and 'b' (see Figure 2.1). On testing days the whole group was moved from its home cage into compartment A. From there individual animals could be given access to the learning compartment (B) by opening door 'a' while keeping door 'b' closed. In the learning compartment (B) subjects were presented with the test, using a touch screen (Ts). After subjects had completed the session they were allowed to enter compartment C via door 'b'. From there they could return to their home cage. This set-up allowed us to use the learning compartment (B) as a lock system between compartment A and the, preferred, home cage. The wire mesh directly in front of the touch screen had been removed to allow free access to the screen. Subjects could have auditory, olfactory and limited visual contact with the group.

The test and the delivery of food rewards were operated by a computer. Rewards were delivered into a small cup centred underneath the touch screen (f, see Figure 2.1). Rewards consisted of a pea or piece of corn combined with either a raisin or a piece of peanut. The experimenter left the room after the task had been started. A video camera mounted above the learning compartment allowed the experimenter to monitor and observe the behaviour of the subjects in an adjacent room.

The first of two daily feedings (Hope Farms 2500 Apenvoeder G.O. 14 mm, monkey chow) was usually postponed until after testing. During the training and testing period fruit could only be obtained as reward in the training or testing procedure. Water was available *ad libitum* in both the home cage and in the test cage.

Both groups received extensive training in the separation and testing procedure before testing began, to ensure that the animals would enter the learning compartment voluntarily. In the final part of training all subjects were given a pre-test discrimination task in which they were familiarised with the

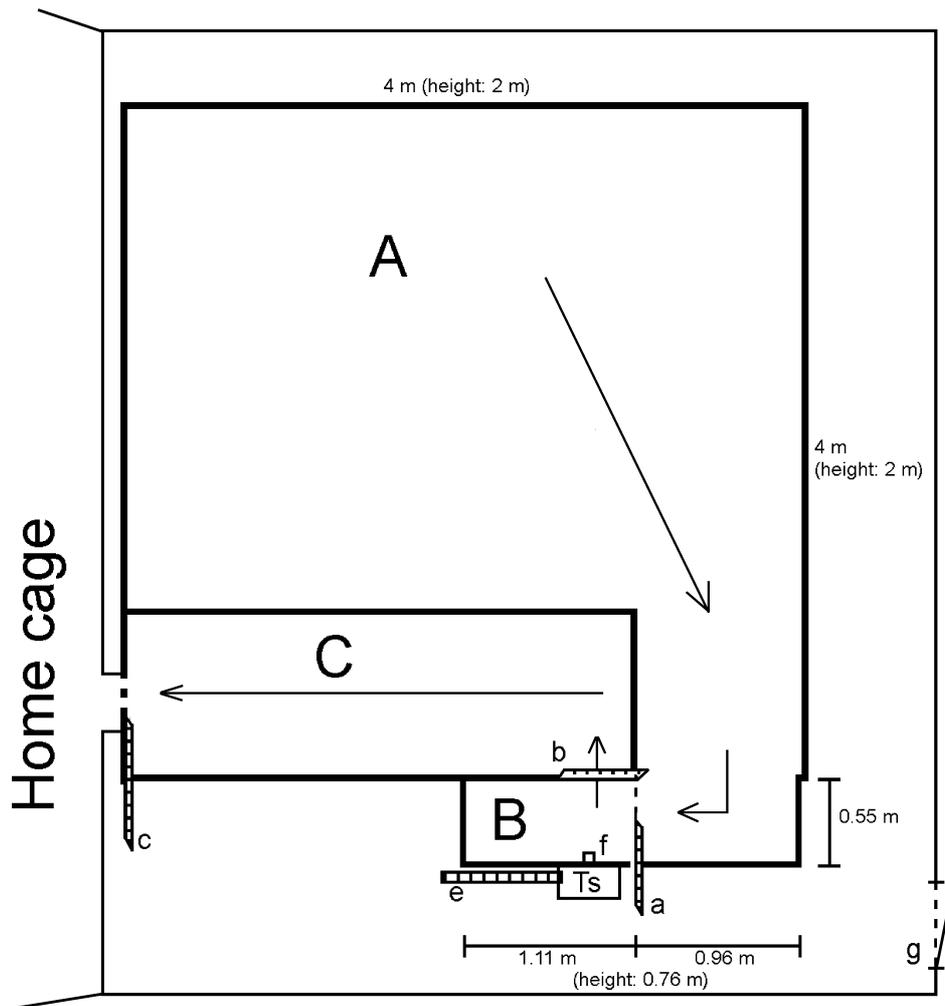


Figure 2.1. Top view of experimental cage. A, B, C: compartments of experimental cage; a,b,c: wooden sliding doors; e: feeder; f: food cup; g: door to enter test chamber; Ts: touch screen. Arrows indicate the test route for the subjects. See text for details. (Adapted from Veenema, 1998)

discrimination test. In this pre-test animals were required to discriminate between a blue hexagonal shape and red oval. A correction paradigm was used such that after a wrong choice the chosen stimulus would disappear only to immediately reappear on the screen. The animal could now only proceed to the next trial after pressing the correct choice. Only trials in which the first response of the animal

was correct counted toward the criterion of 18 correct responses out of 20 consecutive trials. All animals reached this level within seven sessions.

Training and testing of the L-group commenced after testing of the R-group had been completed.

Discrimination-Reversal test

In the present study we used a Discrimination-Reversal (DR) test, which is a widely used test in ageing research. Recent papers have reported variability in performance between aged subjects on this test (Lai et al., 1995; Gallagher & Rapp, 1997; Voytko, 1999; Itoh et al., 2001). This makes the DR-test particularly suitable to investigate possible determinants of this inter-individual variability. The test used was created with Macromedia's Authorware Professional 2.01® for Macintosh. Each trial consisted of the same four pictures being displayed on the four sides of the touch screen. Four, rather than two, pictures were used in order to reduce the number of rewards that could be obtained by responding randomly. The configuration of the pictures was established in a pseudo-random manner such that each picture was displayed equally often in each location within each session of 50 trials. Two of the four pictures showed abstract geometrical shapes, whereas the other two depicted preferred food items situated on a grey, square background (see Figure 5.1). Thus, two stimulus types can be distinguished, namely 'food' and 'abstract'. This was done to examine whether biologically meaningful stimuli would improve performance. Clear differences in performance on the two types of pictures were observed (see Figure 5.2A, and analysis section), however a detailed analysis of these effects extends beyond the scope of this paper.

The animals were given one session each day, seven days a week. When a subject had reached the pre-set learning criterion, which could take several days, the remaining part of the session was cancelled for that day. The following session would start with the previously rewarded picture (correct choice) now being unrewarded (incorrect) and one of the other three stimuli now being rewarded (correct). After such a reversal, errors that were made by responding to the previously correct stimulus (but now incorrect) are termed: perseverative errors. Presenting four pictures allows for a direct analysis of the perseverative tendencies of our subjects.

The learning criterion was set to nine correct choices out of the last ten trials. With chance levels at 0.25 this learning criterion is sufficiently high to ensure that the probability of a subject reaching this learning criterion by chance is below 0.05. The test starts with an initial discrimination task in which the reward association must be learned, next the reward association is switched in a series of reversal tasks.

Measures

The performance of the subjects was measured in two different ways. First, the number of trials a subject needed (trials without a response were omitted) to reach the learning criterion on a certain task is called the *trials to criterion* on that particular task. In our analysis we used a logarithmic transformation of this measure, because the data were not normally distributed.

Secondly, the number of perseverative mistakes a subject made before she reached the learning criterion on a certain task is expressed as a percentage of the total number of mistakes she made on that task. This is called the *percentage of perseverative errors*.

Analysis

All analyses were done using SPSS 10® for Macintosh. All subjects, but one, attained criterion on the initial discrimination task and four consecutive reversal tasks. The subject who did not reach criterion on four reversals (Yukka) consistently responded to one side of the screen for over 2000 trials. Therefore, data of this female were ignored in all analyses concerning the reversals.

Controlling for stimulus type and previous experience

The difference in performance between the two picture types will be the focus of a detailed analysis, reported in Chapter 5. For now, it is important to note the difference in performance between the two types (Figure 5.2A). Evidently, the number of trials to criterion varies as a function of tasknumber and stimulus type. However, no interactions between stimulus type or tasknumber with either experience, age or dominance history was found (data not shown). Therefore, in this paper, we will suffice by controlling for the effects of picture type and task number, and calculating a mean value for each subject. By doing so, we obtain an overall measure of the required trials to criterion and perseverative tendencies of the subjects over these four reversals.

Because six of the subjects in this study also participated in the pilot study we used a T-test to examine if there was a difference in trials to criterion on the reversals between these experienced subjects and the experimentally naïve subjects. This test revealed that experienced subjects needed significantly fewer trials to reach criterion than naïve animals ($t = -3.06$, d.f. = 6.6, $p = 0.02$, two-tailed). Four years after the initial pilot study, experienced subjects still had an advantage over their naïve group members. Therefore, we controlled for experience by including this factor in all analyses.

Statistical tests

This study examines whether long-term social subordination accelerates age-related cognitive decline. The dominance history of our female subjects was taken to indicate prolonged exposure to elevated levels of glucocorticoids. We

examined whether the number of trials to criterion on the initial discrimination task, the reversal tasks and the percentage of perseverative errors were affected by age, dominance history or their interaction. In other words we investigated whether the slope of the age-related decline in performance depends on dominance history. We used General Linear Models with either trials to criterion or perseverative errors as dependent variable and experience and dominance history as fixed factors; age was incorporated as a co-variable. We incorporated experience as a factor in these models, to ensure that possible effects were not due to a difference in experience on such tests. Besides this, we also tested for the presence of an interaction effect between experience and age or between experience and dominance history on each of the three dependent variables. These interaction effects were non-significant and therefore dropped from the model.

In order to compare the results of this study with our pilot study we calculated the standard deviation of the trials to criterion of the subjects in the three age categories in both studies. We tested whether variation in the various age groups was different using a 'Levene's Test for Homogeneity of Variance'.

RESULTS

A summary of the data for each subject is given in Table 2.2.

Initial discrimination task

We first tested for the presence of an interaction effect between age and dominance history on trials to criterion in the initial discrimination task. This interaction turned out to be non-significant ($F(2, 8) = 0.59, p > 0.1$). The slopes of the three regression lines of the effect of age did not significantly differ from each other, depending on the dominance history (Figure 2.2). Therefore, the interaction was dropped from the model. When testing the simpler main effects model, we found that age does not have a significant effect on the trials to criterion ($F(1, 10) = 2.23, p > 0.1$).

The effect of experience approached significance ($F(1, 10) = 3.83, p = 0.079$). Subjects with a history of low dominance performed significantly better on the initial discrimination than animals with a history of high or middle rank ($F(2, 10) = 5.29, p = 0.027$; see Figure 2.2).

So, no age-related decline in trials to criterion on this discrimination task was found, but dominance history did have an influence on the trials to criterion.

Reversals

Trials to criterion

Similarly as above we first tested whether the age-related increase in trials to criterion was more pronounced for subordinate than for dominant animals.

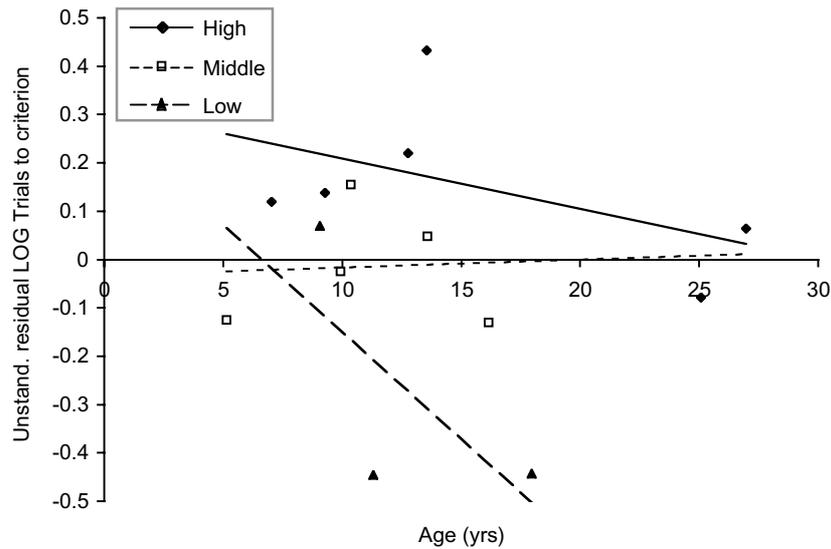


Figure 2.2. Trials to criterion for the initial discrimination of the Discrimination-Reversal test. The residual values are corrected for the effect of experience.

Again, this interaction between age and dominance history turned out to be non-significant ($F(2, 7) = 0.26, p > 0.1$), so was dropped from the model. Testing the main effects model it turned out that experience positively affected the number of trials, whereas age had a negative effect ($F(1, 9) = 20.16, p = 0.002$; $F(1, 9) = 5.19, p = 0.049$ respectively). Note that both these effects are unique effects, meaning that each effect is controlled for the other variable's effect on the number of trials. However, the effect of dominance was not significant ($F(2, 9) = 1.01, p > 0.1$). Figure 2.3 illustrates these results showing that age has a detrimental effect on learning performance.

Perseverative errors

With the average percentage of perseverative errors in the reversal task as dependent variable no interaction effect between age and dominance history was revealed ($F(2, 7) = 0.35, p > 0.1$; Figure 2.3). Testing the model with only the main factors resulted in a significant positive effect of experience ($F(1, 9) = 15.11, p = 0.004$). No effects of age ($F(1, 9) = 0.98, p > 0.1$) or dominance history ($F(2, 9) = 2.15, p > 0.1$) were found.

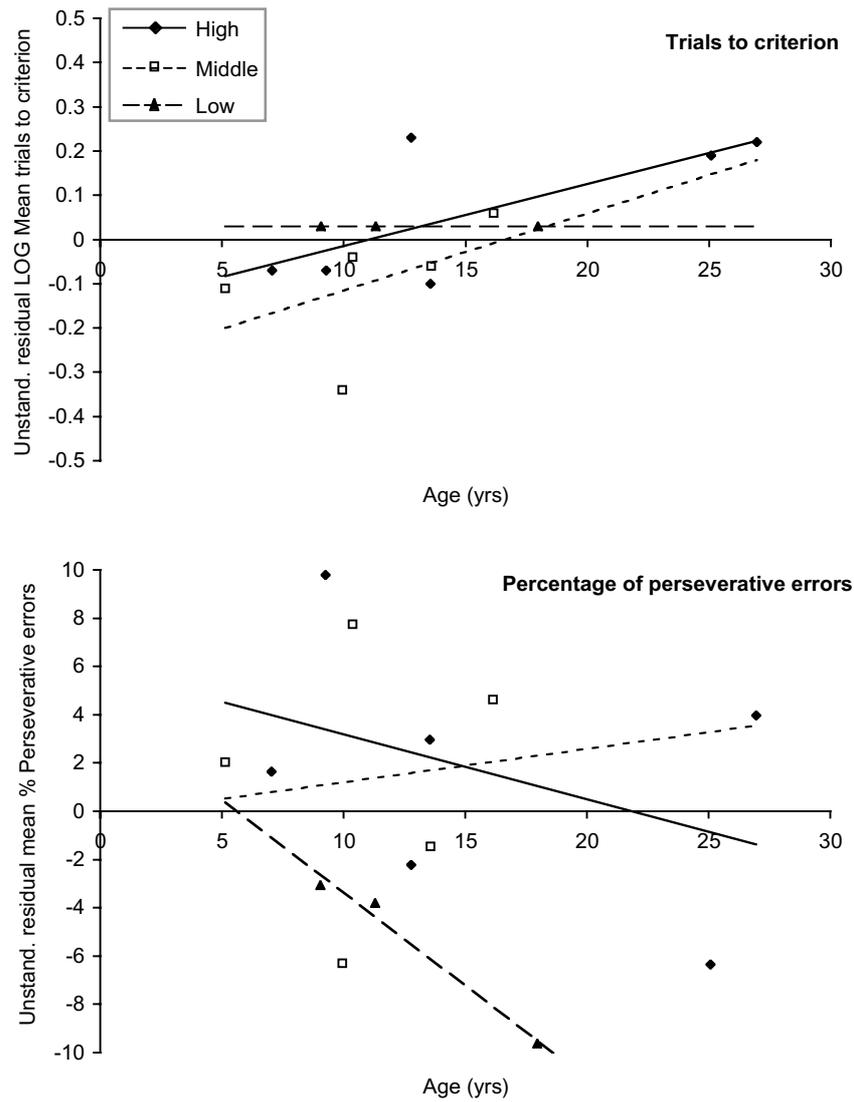


Figure 2.3. Trials to criterion and percentage of perseverative errors for the reversals of the Discrimination-Reversal test. The residual values are corrected for the effect of experience.

Variation & Comparison with pilot study

In this study no accelerating effect of dominance history on age-related cognitive decline was found, which is in contrast with the results from our pilot study. Because of this discrepancy, we were interested to see what the variation was in the age groups of both studies. We calculated standard deviations (s.d.) for the mean number trials to criterion of the subjects in three age categories (Table 2.3). With a 'Levene's test for homogeneity of variance' we tested both studies separately to see whether there were differences in the variance between the age groups. This yielded a p-value approaching significance level for the pilot study ($p = 0.059$). The variance between the age groups in the present data however was far from significant ($p > 0.1$), meaning that the inter-individual variation in the trials to criterion between the age groups was more similar in our study than in the pilot study (Table 2.3). In contrast to our prediction, young animals show a relatively large variation in the present study, while the old animals show a relatively small variation.

Table 2.3. Inter-individual variation in trials to criterion within age categories in the pilot and the current study.

| | Age category | Pilot study | | Current study | |
|---|----------------|-------------|-------|---------------|-------|
| | | N | s.d. | N | s.d. |
| 1 | age < 9.5 | 4 | 0.037 | 4 | 0.058 |
| 2 | 9.5 ≤ age ≤ 14 | 4 | 0.190 | 6 | 0.184 |
| 3 | age > 14 | 4 | 0.202 | 4 | 0.095 |

DISCUSSION

The goal of this study was to examine the hypothesis that age-related cognitive decline is accelerated by a history of low dominance rank. Before discussing the results concerning this hypothesis it is important to see whether this study is in line with existing knowledge on age-related cognitive impairments.

Age-related cognitive decline

Age did not influence the initial discrimination, however, we did find an age-related impairment for the trials to criterion on the reversals. These results are in agreement with the findings of a number of other studies (Bartus et al., 1979; Dean & Bartus, 1988; Moss et al., 1988; Walker et al., 1988; Itoh et al., 2001). Therefore, socially housed monkeys can be used to study age-related cognitive decline.

We did not find an increase in the percentage of perseverative errors with age, which is in contrast with previous studies in which a relation with age was shown (Lai et al., 1995; Voytko, 1999; Itoh et al., 2001). Voytko (1999), however,

reported this age effect only for reversals of an object discrimination, but not for reversals of a more difficult pattern discrimination. The present result could also be related to the type of stimuli used (see Chapter 5). Also, see the discussions below.

The performance of the present subjects is similar to the range of performance of subjects in other studies in which a Discrimination-Reversal test was used with pictorial stimuli (Bartus et al., 1979; Voytko, 1999). Yet, the profound learning effect of aged animals reported by Voytko (1999; see also Anderson et al., 1996) was absent in the present study. This difference in results is likely to be related to the use of two types of stimuli in the present experiment. See Chapter 5 for a more detailed analysis of the effects of these types of stimuli.

Dominance history

No evidence was found for an effect of the dominance history on either the trials to criterion on the reversals or the perseveration tendency. However, we did find that subjects with a history of high dominance rank required significantly more trials to reach criterion on the initial discrimination than subjects with a history of low dominance rank. At first, this result seems to be in agreement with the finding of Bunnell and co-workers (Bunnell et al., 1980) of a relatively poor performance of high ranking male long-tailed macaques in a DR-test compared to lower ranking subjects. However, when we repeated our analysis taking dominance status at the beginning of testing as a factor in stead of the dominance history (due to the rare rank reversal this differed for three out of fifteen subjects) no effect of this present dominance status was found ($F(2, 10) = 2.86, p = 0.10$).

The interaction between ageing and dominance history

Even though the pilot study described here yielded results in line with the hypothesis, the present study provided no evidence for an accelerating effect of dominance history on age-related cognitive decline. No interaction effect between age and dominance history was found for the trials to criterion on the initial discrimination, the reversals or the perseverative errors. Thus, these results give no reason to suggest that a life time of social subordination accelerates age-related cognitive decline.

There are a number of possible explanations for this. For one, the theoretical framework behind the hypothesis may be false. First of all, a low dominance status may not be related to increased exposure to elevated levels of glucocorticoids. Second, prolonged exposure to elevated glucocorticoid levels may not have a detrimental effect on cognitive abilities in the longrun.

Obviously, regular measurements of basal cortisol values throughout the lives of the subjects (i.e. starting in the 1970s) would have been the ideal measure to test our hypothesis with. Unfortunately, no such data were available for these subjects. Furthermore, as we have argued in the introduction there is direct and

indirect evidence from previous studies, supporting both aspects of the theoretical framework behind our hypothesis (Belanoff et al., 2001; Abbott et al., 2003).

Perhaps the hypothesis could not be confirmed, because the psychosocial stress associated with subordination was not sufficiently intense or chronic in our subjects. The social group (most importantly the family members) may have given adequate support to subordinates to compensate the experienced stress, or the chronic nature of the stress. Although social support clearly has an important role in attenuating stress (cf. Von Frijtag et al., 2000), human studies have shown that differences in the amount of social support were related to stress and the rate of cognitive decline (Arbuckle et al., 1992; Antonucci et al., 2000; Seeman et al., 2001; Zunzunegui et al., 2003). Moreover, subordinate primates generally receive more, unpredictable, aggression and have fewer social interactions than dominants. Also, subordinates can exert little control over their environment, and have few outlets for frustrations (Melnick & Pearl, 1987; Sapolsky, 1994; Shively et al., 1997; Shively, 1998; Mendoza et al., 2000). These reports combined with the fact that in long-tailed macaques subordinate families are smaller than dominant families reduces the likelihood of the explanation that adequate social support mitigated social stress in our subjects.

Considering these arguments, it seems premature to falsify our hypothesis based on the present results. It appears to be more fruitful to aim at identifying additional explanations for the found results. Two categories of additional explanations can be distinguished: methodological limitations, and additional factors (such as motivation) that may need to be incorporated into a model describing age-related cognitive decline in primates.

Methodological limitations

Sample sizes are typically small in studies of cognitive capacities of primates (cf. Anderson et al., 1996). Yet, the pilot study resulted in an observed power of 0.71 suggesting that the sample size of the current experiment would be sufficient to detect the interaction effect found in the pilot study.

Nevertheless, a few remarks should be made about the subjects. In this study for some families only one or two females were tested, rather than one female of each age category within every family. This reduced the possibility to control for idiosyncratic differences between the families. Families may differ in, for instance, temperamental aspects for which we could not control by comparing young females with their older family members. Furthermore, in the present study the two oldest subjects, both 25 or more years old, had a history of high dominance rank, and there was a large age difference with the other 'old' subjects. Perhaps, the younger of these old subjects, 18 and 16 years of age, were too young to show a difference in the detrimental effects of prolonged exposure to elevated levels of glucocorticoids, while the other two old subjects both had a history of a dominant position.

The last issue related to the subjects in this study concerns the hypothesis that the psychosocial stress of a life time of low dominance accelerates age-related cognitive decline. This suggests that the effect of subordination has not yet affected the cognitive abilities of young animals. One would therefore expect the variation in the young group to be small in comparison to the aged group. Such an increase in the variability of cognitive performance with the age of subjects has also been reported in humans and primates (Nelson & Dannefer, 1992; Gallagher & Rapp, 1997; Ylikoski et al., 1999). In the current study, however, this variation was similar in both the young and aged group (see Table 2.3 and the discussion below). The relatively small variation among the aged animals may be related to the next issues.

Also, we have to consider the number of stimuli used in this experiment. All previous studies that have examined DR performance (including our pilot study) have presented their subjects with two stimuli. In the current study subjects had to choose between four two-dimensional stimuli. The main reason for this set-up was to increase motivation to engage in the task by reducing the number of rewards that could be gained by responding randomly. However, by presenting four stimuli we may have made the DR-test too difficult. In order to examine this possibility we compared the average number of trials to criterion of the naïve subjects of this study with the subjects of the pilot study who were not also part of this study. We performed a T-test in which showed that the subjects of this study indeed needed significantly more trials to reach the learning criterion than the subjects of the pilot study ($t = -3.62$, $d.f. = 13$, $p < 0.01$, two-tailed). Possibly, the DR-test used here was too difficult for a differentiating effect of life time subordination on age-related cognitive decline.

Finally, the analysis as it is presented here may have reduced the possibility of finding differences. As we focussed on the four reversals on which all subjects managed to reach the learning criterion, we neglected the learning that occurred in the animals that reached criterion on more than four reversals, in the same time span as the slowest learners completed only those four reversals. Thus, we may have a greater chance of finding a differentiating effect of life time dominance on age-related cognitive decline if we analyse overall performance of subjects on a set period of time, in which some will have reached criterion on more reversals than others. Nevertheless, such an analysis (for more information see Chapter 3) did not yield different results with respect to the predicted interaction effect of life time dominance history and age (data not shown). Obviously, the variation in results increased; for the aged group it now reached a level similar as in the pilot study. However, the variation among young animals was much larger in this analysis than in the pilot study (Table 2.4). This relatively large variation in the young animals, and the large variation in perseverative mistakes (Figure 2.3), suggests that other factors may have played an important role in determining performance.

Table 2.4. Inter-individual variation in trials to criterion within age categories, for the two stimulus types.

| | Age category | N | Abstract | Food |
|---|----------------|---|----------|-------|
| | | | s.d. | s.d. |
| 1 | age < 9.5 | 4 | 0.104 | 0.175 |
| 2 | 9.5 ≤ age ≤ 14 | 6 | 0.233 | 0.179 |
| 3 | age > 14 | 4 | 0.208 | 0.207 |

Values are based on average performance measured over a similar time span for all subjects. For statistical reasons the two types of stimuli were analysed separately.

Possible confounding factors

Non-human primates are considered to be a valuable model in the study of the ageing process. Usually, the only factor determining cognitive capacity in this model is the calendar age of an individual. Human ageing research, however, has made clear that age-related cognitive decline is also influenced by a number of other factors such as biological life events (Jolles et al., 1993), education and prolonged exposure to glucocorticoids. Additionally, factors such as motivation (Tomporowski & Tinsley, 1996), acute stress (Lupien & McEwen, 1997) and temperamental differences (Arbuckle et al., 1986; Mueller, 1992; Persson et al., 2000) may influence human cognitive performance.

We have argued that life time dominance history is likely to be a factor that should be incorporated in the primate model. Calendar age and life time dominance history, mediated by prolonged exposure to elevated glucocorticoids levels, together determine cognitive capacity. However, it is obvious that also in primates additional factors may also be at play in determining performance on a cognitive test. Yet, little knowledge exists concerning the influences of factors, such as motivation, on primate cognitive performance. Future studies could address the aspects of acute stress and motivation on cognitive performance in relation to the social environment as suggested by Bunnell et al. (1980).

Additionally, individual differences in aspects of temperament, such as trait anxiety, could directly affect cognitive performance (Schneider et al., 1991; Mueller, 1992; Ohl et al., 2003), and determine how subjects behave in a testing situation (Itoh, 2001). Furthermore, such differences in temperament have been related to differences in basal levels of glucocorticoids (Kalin et al., 1998; Byrne & Suomi, 2002). Thus, it is likely that such individual characteristics can reduce or enlarge the impact of long-term stress, acute stress, and influence the motivation of subjects to engage in cognitive tasks. Therefore, temperament is an important factor to be included in the primate model of age-related cognitive decline.

CONCLUSIONS

Our results are in line with existing literature concerning the age-related deficit in performance on a DR learning test. However, this study found no evidence for an acceleration of age-related cognitive decline in relation to a life time of social subordination. A number of methodological limitations, regarding the sample size and the number of stimuli that were used, are raised. Still, we argue that the socially housed primate can be a valuable model to study age-related deficits in cognition, but that this model should be considered in more detail. Additional factors besides calendar age and life time dominance status may influence performance on cognitive tests. These factors should be taken into account for a proper interpretation of the data, especially when investigating social influences on age-related cognitive decline.

ACKNOWLEDGEMENTS

We thank Michelle Doumen, Patricia Verschoor and Leonie de Visser for assisting in the data collection. Henk Westland and Annet Louwerse provided practical assistance. Han de Vries and Miranda Swagemakers gave statistical advice and helpful comments.

CHAPTER 3

Effects of attention, arousal and dominance on performance of socially housed monkeys in a learning test

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ABSTRACT

Socially-mediated factors which are known to influence cognitive performance in humans have traditionally been neglected in studies using primates. This study examined the influence of behavioural arousal and attention, as indications of acute stress and motivation to engage in the task, on the performance of fourteen socially housed female monkeys (*Macaca fascicularis*). Dominance status, age, and previous experimental experience of subjects were recognised as factors that could affect arousal, attention and performance on a Discrimination-Reversal test. Both high arousal and low attention were expected to be related to high dominance status and reduced performance. Previous experimental experience was predicted to be associated with low arousal.

Results show that low dominance status was associated with high levels of attention. Furthermore, a high level of attention was related to good performance. In contrast to the expectations experienced subjects showed high levels of arousal; arousal was not related to performance. The results indicate that these socially-mediated variables are as important to consider in primate studies of cognition as they are in human studies.

INTRODUCTION

This paper deals with the relation between social dominance status, motivation to engage in a cognitive task, acute stress during this task, and individual performance of socially housed monkeys (*Macaca fascicularis*).

Non-human primates have been studied intensively as the best model for human cognitive functioning (reviews: Ridley & Baker, 1991; Price et al., 1994; Gallagher & Rapp, 1997; Voytko, 1997). Most studies have looked exclusively at one factor, i.e. age, although recent papers on human cognitive performance have demonstrated that cognition cannot be considered in isolation. Human cognitive performance is affected by a wide range of factors, including age, long-term stress, social support, temperament, motivation, acute stress and anxiety (Arbuckle et al., 1992; Lupien et al., 1994; Albert et al., 1995; Filipp, 1996; Tomporowski & Tinsley, 1996; Lupien et al., 1997; Seeman et al., 1997; Evers et al., 1998; Bassuk et al., 1999; Newcomer et al., 1999; Persson et al., 2000; Seeman et al., 2001; Wolf, 2003).

Another aspect of tests of primate cognitive abilities is that, traditionally, the animals used were solitary housed, with disregard for the natural social life of the monkey species used in these studies. This social environment is, however, essential for normal emotional and cognitive functioning in primates (Suomi, 1991a; Sanchez et al., 1998; Sanchez et al., 2001; Lutz et al., 2003). Furthermore, it has been reported to influence cognitive functioning in both monkeys and humans (Monteil & Huguet, 1993; Veenema et al., 1997; Seeman et al., 2001; Veenema et al., 2001; Zunzunegui et al., 2003).

In a previous study the influence of long-term social subordination was examined, as one aspect of the social environment that may have a differentiating effect on cognitive capacity among old socially housed monkeys (Chapter 2). That study suggested that other, immediate factors, such as acute stress, affected the performance of subjects. The present paper, therefore, assesses the immediate influence of: 1) motivation to engage in the task, 2) acute stress and 3) dominance status on the cognitive performance, as measured in the earlier study.

Both motivation and acute stress were reported to directly affect cognitive performance in humans (Eysenck, 1985; Perlmutter & Monty, 1989; Filipp, 1996; Kirschbaum et al., 1996; Tomporowski & Tinsley, 1996; Lupien et al., 1997; Newcomer et al., 1999; Hess et al., 2001; al'Absi et al., 2002; Wolf, 2003; West & Yassuda, 2004). For primates little is known about the influence of these factors on cognitive performance. Arnsten & Goldman-Rakic (1998) reported that acute stress negatively affected cognitive performance. Bunnell and colleagues (Bunnell et al., 1980; Bunnell & Perkins, 1980) reported that the performance of male long-tailed macaques (*Macaca fascicularis*) was negatively affected by their rank position. They suggested that this was a side effect of the "chronic social pressure" (p. 387, Bunnell et al., 1980) of maintaining a high dominance rank (see also Nash

& Chamove, 1981). From their results it was not clear whether this effect was related to either acute stress, or reduced motivation to engage in the task for other reasons such as a low motivation to obtain food rewards. In the present study we test whether high dominance status is related to both high levels of acute stress and low levels of motivation to engage in the task. Moreover, acute stress and motivation to engage in the task are expected to be associated with a relative reduction or improvement of performance, respectively.

Unfortunately, motivation and acute stress are difficult to assess in cognitive tests with group-living primates. Motivation has been quantified as the willingness to repeatedly respond to a stimulus for a reward (Weed et al., 1999). In a cognitive task, however, such a paradigm to assess motivation is not feasible. Instead, a behavioural measure of attention is used to indicate motivation to engage in the task. Attention was measured as the relative amount of time the animals attend to the test. In view of the suggestions of Bunnell and co-workers (1980), we expect that a high dominance status will be related to reduced attention and performance on the cognitive test. In the human literature differential results are reported for the effect of age on sustained or divided attention or motivation (e.g. Tomporowski & Tinsley, 1996; McDowd & Shaw, 2000; Hess et al., 2001; Hogan, 2003). The very general measure of attention used here is difficult to relate to these measures. Therefore, we make no explicit predictions about the relation between age and the measure of attention used here.

Similarly, a behavioural measure of arousal is used as an indication of acute stress. The occurrence of certain behaviours (such as scratching) has been suggested to be a behavioural indication of the emotional arousal underlying anxiety, uncertainty and elevated levels of glucocorticoids (Pavani et al., 1991; Maestripieri et al., 1992; Schino et al., 1996; Troisi et al., 1996; Leavens et al., 1997; Troisi, 2002). On the other hand, such emotional arousal may also have a more positive basis such as excitement in anticipation of positive experiences (Lang, 1995; Thayer, 1996; Spruijt et al., 2001; Merali et al., 2004). Furthermore, elevated arousal and levels of glucocorticoids may have either a negative or positive influence on cognitive performance depending on their level, according to an inverted U-shaped function (Diamond et al., 1992; Mueller, 1992; Lupien & McEwen, 1997). These considerations call for a cautious interpretation of displacement behaviours in terms of anxiety. We, therefore, regard displacement behaviours as a neutral and general indication of emotional arousal. Clearly, arousal comprises a rise in general, aspecific alertness to (changes in) the surroundings of the individual. This general alertness is different from the specific attention to the task described above. In the context of the present experimental situation we expect this behavioural arousal to find its origin in acute stress, due to the separation procedures used in this study.

The work described here was part of a larger study concerning intervening factors on age-related cognitive decline. A Discrimination-Reversal (DR) learning

test was used to examine the learning abilities of socially housed long-tailed macaques (*Macaca fascicularis*). In this DR-test two kinds of stimuli were used, namely abstract and realistic, familiar (that is 'food') stimuli. Elsewhere, we deal with the hypothesis that familiar (i.e. food) stimuli are more easily discriminated than unfamiliar (abstract) stimuli (see Chapter 5). In the present paper we will analyse the results of these two kinds of stimuli separately for statistical reasons only. Previously described results from this DR-test showed that cognitive performance was associated with increased age and prior experimental experience of the subjects (Chapter 2). Therefore, the age and experience of the subjects will be incorporated in the present analysis.

In summary, we predicted the following relations in our model (Figure 3.1). We expected that subjects with a high level of behavioural arousal and a low level of attention would show a reduced performance compared to other subjects. Additionally, we expected that low levels of attention for the task would be related to high dominance status. Likewise, high levels of behavioural arousal were hypothesised to be related to high dominance status. Finally, the separation procedure used here was predicted to arouse the subjects. Yet, experienced subjects are expected to be less aroused by these procedures than naïve animals.

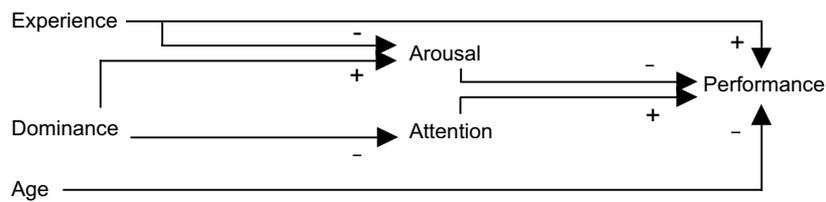


Figure 3.1. Representation of the authors' proposed relations between the factors studied here. A '+' indicates a positive relation: a high level in one factor is related to a high level in the other. A '-' indicates a negative relation: a high level in one factor is related to a low level in the other. A high level of performance means that few trials to criterion were needed and a low percentage of perseverative errors was made.

METHODS

Subjects

This experiment was part of a larger study concerning age-related cognitive decline of female monkeys (see Chapter 2). Two groups of socially housed

Table 3.1. Group, name, dominance rank, age, experience, testing period, performance and behavioural data for the subjects

| Group | No. | Name | Rank ¹ | Age ² | Exp ³ | DR test period | | | Abstract reversals | | | Food reversals | | | |
|-------|-----|----------|-------------------|------------------|------------------|-------------------|-------------------|---------------------|---------------------|---------------------|--------------------|---------------------|---------------------|---------------------|--------------------|
| | | | | | | Days ⁴ | Revs ⁵ | Trials ⁶ | Persev ⁷ | Attent ⁸ | Arous ⁹ | Trials ⁶ | Persev ⁷ | Attent ⁸ | Arous ⁹ |
| R | 1 | Felix | 1 | 27.0 | Yes | 55 | 7 | 200.8 | 62.4 | 61.2 | 0.49 | 414.3 | 35.5 | 71.2 | 0.32 |
| R | 2 | Alfa | 1 | 12.8 | Yes | 49 | 5 | 185.7 | 59.1 | 38.2 | 1.01 | 468.0 | 31.1 | 37.7 | 0.65 |
| R | 3 | Kraa | 1 | 9.3 | Yes | 56 | 21 | 71.6 | 58.2 | 49.8 | 0.81 | 111.6 | 49.6 | 56.8 | 0.91 |
| R | 4 | Milva | 2 | 10.4 | No | 56 | 9 | 158.3 | 71.4 | 77.8 | 0.41 | 314.4 | 43.6 | 65.5 | 0.26 |
| R | 5 | Yudea | 2 | 13.6 | Yes | 56 | 17 | 65.1 | 54.8 | 88.4 | 0.80 | 190.7 | 30.4 | 90.5 | 0.33 |
| R | 6 | Freya | 2 | 9.9 | Yes | 58 | 23 | 40.9 | 48.6 | 86.7 | 0.46 | 146.7 | 21.3 | 79.7 | 0.33 |
| R | 7 | Rastafa | 2 | 5.1 | No | 56 | 9 | 122.5 | 70.0 | 81.7 | 0.20 | 383.6 | 48.9 | 84.0 | 0.22 |
| R | 8 | Roza | 3 | 18.0 | No | 66 | 7 | 171.7 | 60.6 | 81.6 | 0.73 | 613.0 | 28.1 | 82.6 | 0.29 |
| R | 9 | Hoeba | 3 | 11.3 | Yes | 54 | 15 | 71.9 | 54.6 | 62.7 | 1.78 | 212.3 | 26.9 | 62.5 | 0.86 |
| L | 10 | Lixa | 1 | 25.1 | No | 68 | 3 | 400.0 | 58.4 | 19.1 | 0.24 | 876.0 | 43.3 | 57.4 | 0.18 |
| L | 11 | Ikea | 1 | 13.6 | No | 56 | 7 | 206.7 | 59.8 | 41.7 | 0.31 | 355.5 | 47.2 | 56.8 | 0.30 |
| L | 12 | Virginia | 1 | 7.0 | No | 60 | 6 | 198.3 | 60.3 | 30.5 | 0.11 | 440.7 | 46.0 | 37.4 | 0.11 |
| L | 13 | Bilboa | 2 | 9.1 | No | 58 | 6 | 211.0 | 65.4 | 49.0 | 0.06 | 581.0 | 32.6 | 60.9 | 0.03 |
| L | 14 | Kaa | 3 | 16.1 | No | 56 | 3 | 228.0 | 70.9 | 35.6 | 0.17 | 312.5 | 57.8 | 14.2 | 0.14 |
| L | 15 | Yukka | 3 | 7.0 | No | 64 | 1 | | | | | | | | |

¹ Present dominance rank category (see text for details)

² Age in years

³ Indicates if the animal had previous experience

⁴ Test period in days (see text for details)

⁵ Number reversals on which the learning criterion was reached (see text for details)

⁶ Trials: mean number of trials to criterion

⁷ Perseverative errors: mean percentage of perseverative errors

⁸ Level of attention: mean percentage of time subjects attend the test

⁹ Arousal: mean rate of displacement behaviours

¹⁰ Data for Yukka were omitted from analyses of the reversals

long-tailed macaques (*Macaca fascicularis*) participated in this study, the R- and L-group. Both groups were housed at the Ethology Station of Utrecht University; for more information on the groups and their housing see Chapter 2. Fifteen female subjects of different age and dominance status were selected: nine from the R-group and six from the L-group.

The subjects were divided into three 'present' dominance rank categories (Table 3.1), signifying whether females occupied a high dominance rank (1), a middle dominance rank (2) or a low dominance rank (3) in their group at the start of testing. All members of both groups were known individually since birth. Also, the dominance relations between families within the groups have been monitored on a regular basis, based on unidirectional submissive behaviour (Angst, 1974). The dominance categories were based on this information.

Six of the selected females from the R-group had previously participated in a pilot study four years earlier (Veenema, 1998), and will be considered as 'experienced' subjects. Relevant characteristics for the subjects are given in Table 3.1.

Experimental set-up and testing procedure

Testing was conducted in an experimental wire mesh cage situated in a room adjacent to the animals' home cage. The experimental cage contained a learning compartment in which subjects could be separated from the group (auditory, olfactory, and limited visual contact remained possible). The learning compartment could be used as a lock system between the experimental cage and the home cage. For more information on this set-up and the testing procedure see Chapter 2 and Figure 2.1.

In the learning compartment subjects were presented with the test, using a touch screen. The test and the delivery of food rewards were operated by a computer. Rewards were delivered into a small cup centred underneath the touch screen.

The first of two daily feedings (Hope Farms 2500 Apenvoeder G.O. 14 mm, monkey chow) was postponed until after testing. During the training and testing period fruit could only be obtained as a reward in the training or testing procedure.

The subjects were given a piece of apple or banana when they entered the learning compartment and after they completed their session. This was done to keep the subjects motivated to enter the learning compartment, even after performance had been low and few rewards had been received. On each trial one reward could be obtained, consisting of a pea or piece of corn combined with either a raisin or a piece of peanut. There was a 25 % chance of obtaining a reward on any trial when responding randomly (see below). The animals were given additional pieces of apple or banana upon reaching the pre-set learning criterion, in order to keep them motivated. Water was available ad libitum in both home- and test cage.

The experimenter left the room after starting the task for a subject. A video camera mounted above the learning compartment allowed the experimenter to monitor and observe the behaviour of the subjects from an adjacent room. Here the behavioural elements displayed by the subjects as they were performing in the cognitive task, were directly logged into a computer file using the Observer® programme.

Both groups received extensive training in the separation and testing procedure before testing began to ensure that the animals would enter the learning compartment voluntarily. In the final part of training all subjects were given a pre-test discrimination task in which they were familiarised with the discrimination test.

The cognitive task used was a Discrimination-Reversal (DR) learning test in which subjects were required to choose one stimulus out of an array of four, namely two of each stimulus type (see above). Subjects had to learn which stimulus was consistently associated with a reward (for more details on this test, see Chapter 2). When this initial discrimination task had been learned, up to the pre-set learning criterion (nine correct out of ten consecutive trials), the association with the reward was switched (reversed) to one of the other stimuli. Such a reversal was done each time a subject reached the learning criterion. Subjects received a maximum of 50 trials per day and could use multiple days to reach the pre-set learning criterion. Training and testing of the L-group commenced after testing of the R-group had been completed. Three subjects of the L-group (Kaa, Yukka and Bilboa) did not respond to any trials when confined to the learning compartment, they were, therefore, tested with more space at their disposal (with access to compartment C of the experimental cage, see Figure 2.1).

Measures

Performance measures: number of trials and percentage of perseverative errors

The performance of the subjects was measured in two different ways. First, the number of trials a subject required (trials without a response were omitted) before reaching the learning criterion on a certain task is called the *number of trials* on that particular task. An averaged logarithmic transformation of this measure was used, because the data were not normally distributed.

Secondly, the number of perseverative mistakes a subject made before reaching the learning criterion on a reversal task is expressed as a percentage of the total number of mistakes she made on that task. This is called the *percentage of perseverative errors*. We calculated an average percentage of perseverative errors for each subject.

Behavioural measures: attention and arousal

We defined the behaviour 'attention' as the subject being within 30 centimetres (approximately one arm's length) of the screen and with its face oriented

towards the screen. For each task (both initial discrimination and reversals) we calculated the time an animal spent in attention as a percentage of the total time an animal was being tested. The average *level of attention* for each subject was used as an indication of the motivation to engage in the test.

We took the rate of displacement behaviours of the subjects as a behavioural indication of the arousal they experienced during testing. We calculated the summed rate per minute of testing time of the occurrence of the following behaviours: scratching, yawning, body shaking, barbiting and self-grooming. An averaged logarithmic transformation of this behavioural *arousal* was used, because the data were not normally distributed.

Analysis

The aim of this study was to investigate the relations between dominance, the level of attention, behavioural arousal and performance in a model of age-related cognitive decline (Figure 2.1). In accordance with previous reports, age did not influence performance on the initial discrimination (see Chapter 2). Therefore, the initial discrimination was omitted from the present analysis.

Studies of primate learning abilities that use a DR-test usually base their analysis on an identical number of reversals completed by all subjects. Applying this approach to our data would result in different test periods for the subjects. Because the occurrence of the behavioural elements fluctuated over time, this approach would result in less reliable averages for attention and arousal for subjects requiring less trials (i.e. less time) to complete reversals. Rather, we decided to take the maximum test period for each subject, while at the same time we minimised the range of testing periods of the subjects. This resulted in the test periods reported in Table 3.1. Evidently, this resulted in a different number of completed reversals (Table 3.1) for different subjects. The average performance measures should, therefore, be considered as a general indication of their cognitive performance, including learning effects, which may be larger for subjects completing more reversals.

On the reversals, one of the subjects (Yukka) consistently responded to one side of the screen for over 2000 trials. The data of this female were ignored in all analyses.

As the DR-test used here involved four stimuli of two kinds (two 'abstract' stimuli and two 'food' stimuli, for more information see Chapters 2 and 5) we analysed abstract- and food reversals separately. The two behavioural measures, arousal and attention, did not correlate (data not shown) and were also analysed separately. All these separate analyses were done using the same scheme of subsequent General Linear Models (GLMs).

In addition to the factors for which predictions were formulated (arousal, attention, present dominance status and experience) we took one characteristic of the subjects, namely their age, into account in our analysis. Previously we have

reported that age affects performance in this experiment (Chapter 2). In all GLMs we included dyadic interaction terms in separate runs of the model, only interactions with a p-value less than 0.10 are reported here. Note that significant interaction effects do not allow interpretation of a separate term of the interaction without reference to the other term of the interaction. Therefore, in the case that interaction terms were found to reach significance, the model was not tested with only the main effects of the variables. However, if none of these interactions proved to be significant, we analysed a model with only the main effects. Due to statistical limitations not all factors under study here could be incorporated simultaneously in the models. The following GLMs were used:

A Effects of experience, dominance and age on performance measures

We used a General Linear Model (GLM) with either the number of trials or perseverative errors as dependent variable, and experience and present dominance status as fixed factors; age was incorporated as a co-variable. This analysis allowed us to control for these effects when, later, examining the effects of attention or arousal.

B Relations with behavioural measures

I Effects of experience, dominance and age on behavioural measures

For this we used a GLM with either the level of attention or arousal as dependent variable, and experience, present dominance status (fixed factors) and age (co-variable) as independent variables.

II Effects of behavioural measures on trials to criterion

We performed a GLM with the trials to criterion as dependent variable and either one of the behavioural measures as co-variable. Next, we incorporated the variables experience, present dominance status as fixed factors and age as a co-variable in this GLM, provided that these variables had a significant effect on the trials to criterion.

III Effects of behavioural measures on the perseverative errors

Similarly, we performed a GLM with either one of the percentage of perseverative errors as dependent variable and either one of the behavioural measures as co-variable. Next, we incorporated the variables experience, present dominance status as fixed factors, and age as a co-variable in this GLM, provided that these variables had a significant effect on the percentage of perseverative errors.

RESULTS

In this study the relation between experience, dominance status and differences in the amount of attention, arousal and individual cognitive performance was examined. For a summary of the data see Table 3.1. Here, a brief report of the results is presented for reasons of clarity. Only the most relevant effects are given, for a full account of the steps taken in the analysis and the effects see the appendix at the end of this chapter.

A Effects of experience, dominance and age on performance measures

We analysed whether experience, age, and present dominance status affected performance of our subjects. Experienced subjects required significantly fewer responses to reach the learning criterion (i.e. had a better performance) than naïve subjects (Abstract: $F(1, 9) = 29.92$, $p < 0.001$; Food: $F(1, 9) = 11.64$, $p = 0.008$). Moreover, old age was associated with more trials to criterion on abstract reversals ($F(1, 9) = 7.39$, $p = 0.024$); this effect approached significance on food reversals ($F(1, 9) = 4.10$, $p = 0.074$). Dominance status did not further effect the number of trials to criterion.

With respect to the perseverative errors a significant interaction effect between experience and dominance status ($F(2, 7) = 7.38$, $p = 0.019$) complicated interpretation of the results for abstract reversals. Further inspection showed that this interaction effect was primarily due to a difference between experienced and naïve animals in the middle dominance category (data not shown). When the interaction effect was omitted from the model, only experience had a significant negative influence on the perseverative tendencies ($F(1, 9) = 6.41$, $p = 0.032$). For food reversals a similar, but not significant, effect of experience was found ($F(1, 9) = 4.86$, $p = 0.055$).

So, the number of trials decreased significantly with experience and increased with age for both types of stimuli (see above). Moreover, the percentage of perseverative errors was lower for subjects with previous experience. Additionally, dominance status may also affect the perseverative errors. Therefore, in the following analysis of the influence of the behavioural factors on performance we will control the effects on trials to criterion for experience and age. The effects on the perseverative tendency will be corrected for experience and dominance status.

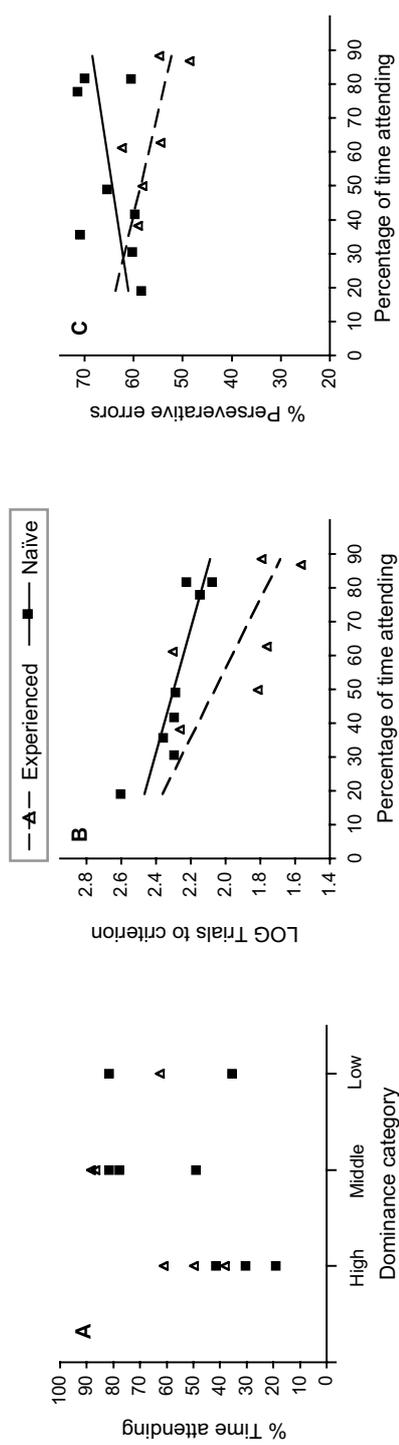


Figure 3.2. Level of attention in relation to present dominance rank (A) and the performance measures (B, C) for the abstract reversals

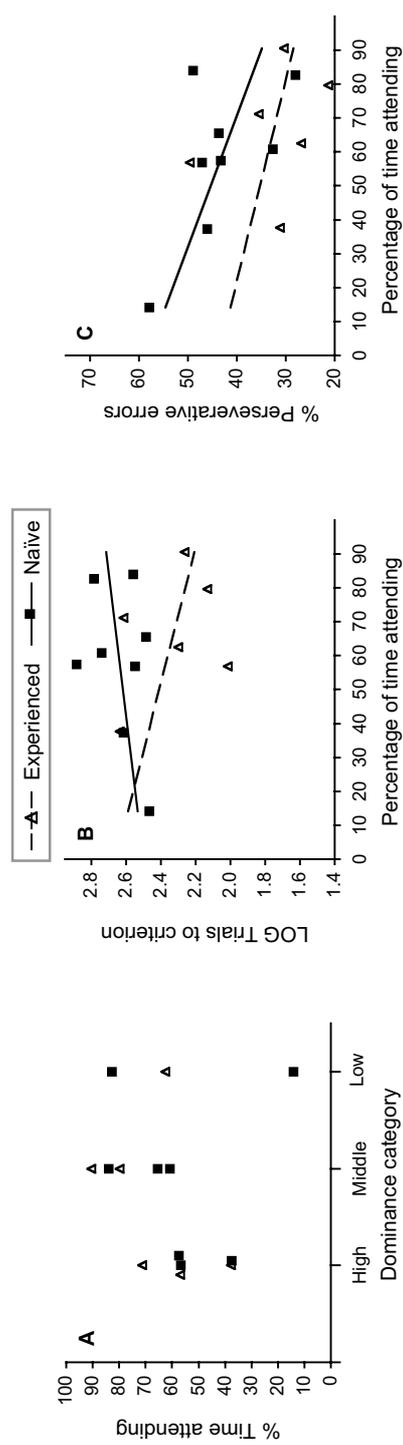


Figure 3.3. Level of attention in relation to present dominance rank (A) and the performance measures (B, C) for the food reversals

B Relations with behavioural measures

Level of attention

I Effects of experience, dominance and age on the level of attention

We first examined how experience, dominance status and age influenced the level of attention. The highest ranking subjects showed the lowest level of attention on abstract reversals ($F(2, 9) = 6.90$, $p = 0.015$; see Figure 3.2A). Analysis of the food reversals revealed a tendency in the same direction ($F(2, 9) = 3.09$, $p = 0.095$; Figure 3.3A). Neither age nor experience further affected the level of attention.

II Effects of the level of attention on the number of trials

Subjects with a high average level of attention needed significantly fewer trials to reach criterion on abstract reversals than subjects with a low level of attention ($F(1, 9) = 19.59$, $p = 0.002$; Figure 3.2B), when controlling for experience and age. However, the level of attention did not affect the number of trials to criterion in food reversals (Figure 3.3B).

III Effects of the level of attention on the perseverative errors

On abstract reversals, experienced subjects appear to make fewer perseverative errors when they show more attention, while this is not true for naïve subjects ($F(1, 8) = 5.35$, $p = 0.050$; Figure 3.2C). On food reversals a high level of attention was significantly related to fewer perseverative errors ($F(1, 12) = 5.04$, $p = 0.044$; Figure 3.3C). However, this latter effect did not maintain significance over and above the effects of experience and dominance status. So there is no clear indication that the level of attention reduces the perseverative tendency of our subjects.

Arousal

I Effects of experience, dominance and age on arousal

Experienced subjects showed a higher level of behavioural arousal on both abstract ($F(1, 9) = 13.48$, $p = 0.005$; Figure 3.4A) and food (Experience: $F(1, 9) = 10.41$, $p = 0.010$; Figure 3.5A) reversals. Neither present dominance status, nor age further influenced the behavioural arousal of the subjects.

II Effects of arousal on the number of trials

The initial GLM testing the effect of behavioural arousal on the number of trials revealed that a high level of behavioural arousal was associated with fewer

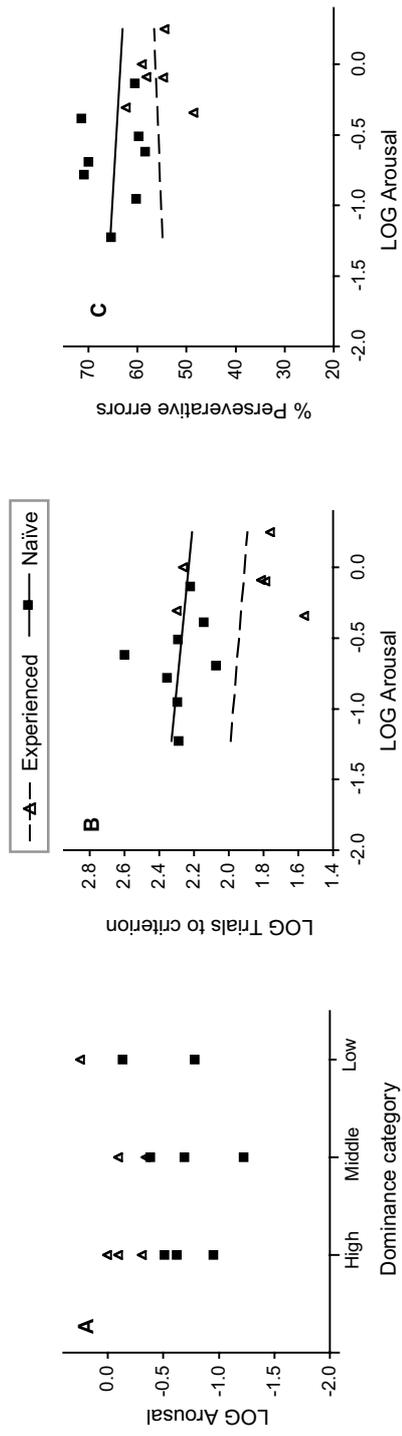


Figure 3.4. Arousal in relation to present dominance rank (A) and the performance measures (B, C) for the abstract reversals

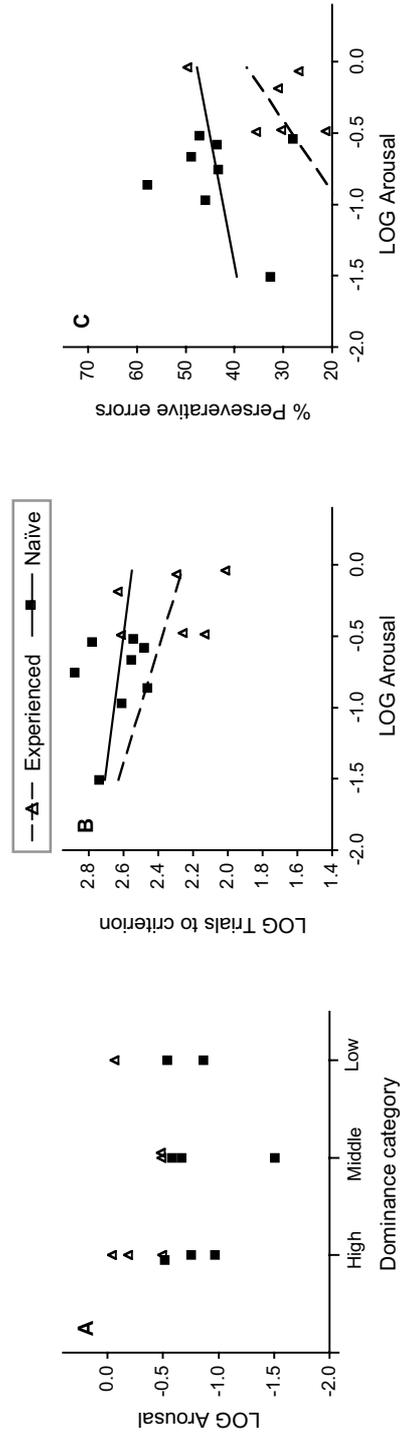


Figure 3.5. Arousal in relation to present dominance rank (A) and the performance measures (B, C) for the food reversals

numbers of trials to reach criterion (Abstract: $F(1, 12) = 4.53, p = 0.055$; Food: $F(1, 12) = 5.23, p = 0.041$). In accordance with the strategy outlined in the Analysis section, experience and age were incorporated in the next GLM to investigate whether the level of arousal had an additional effect over and above the effect of these variables. No additional effects of behavioural arousal on the number of trials were revealed for either abstract or food reversals (Figure 3.4B, Figure 3.5B). So, previous experimental experience was related to both a low number of trials and a high level of arousal. It is unclear whether behavioural arousal influenced the number of trials, or whether these variables correlate, as they are both affected by experience.

III Effects of arousal on the perseverative errors

When only the behavioural arousal was tested in a GLM no significant relation between arousal and perseverative tendency was found (Abstract: $F(1, 12) = 3.93, p = 0.071$; Food: $F(1, 12) = 0.46, p > 0.1$). Similarly, behavioural arousal was not related to the percentage of perseverative errors in addition to the effects of experience and dominance status (Figure 3.4C, Figure 3.5C). So, experience was related to both a low perseverative tendency and a high level of arousal, but arousal was not directly related with perseveration.

DISCUSSION

In this paper we examined the relation between experience, present dominance status, the level of attention, arousal and individual cognitive performance of socially housed long-tailed macaques. We proposed a model, see Figure 3.1, suggesting an intermediate position of attention and behavioural arousal between social dominance and performance. In our analyses we treated the two kinds of reversals (abstract and food) and the two performance measures separately for statistical reasons. In this section, however, we will present a combined discussion.

In summary, we found that: (1) previous experimental experience was related to improved performance; (2) old age was associated with reduced performance; (3) high dominance status was related to low levels of attention; (4) high levels of attention were related to improved performance; (5) previous experience was related to high levels of behavioural arousal; (6) high levels of behavioural arousal may be related to improved performance, but this may also be a covariation in relation to experience.

We will first discuss the results concerning the level of attention of the subjects. Next, the results concerning behavioural arousal will be discussed.

Level of Attention

It was hypothesised that the level of attention for the test would be negatively influenced by high dominance. Additionally, a low level of attention was

expected to be related to reduced performance. Our results confirm these hypotheses.

First, the level of attention positively affected the performance of our subjects. We argued that the amount of attention for the test (operationalised as the time the subject is oriented toward the screen) could be seen as an expression of the subjects motivation to engage in the test (for a relation between sustained attention and motivation in humans see Tomporowski & Tinsley, 1996). When considered in this light, these results are in line with the human literature, where it is reported that a decrease in motivation results in reduced performance on cognitive tests (Perlmutter & Monty, 1989; Filipp, 1996; Tomporowski & Tinsley, 1996; Hess et al., 2001).

Secondly, the highest ranking subjects tended to show lower levels of attention for the test than lower ranking individuals. Both these results concerning the relations between social dominance, attention and performance are in line with previous suggestions in the primate literature. Bunnell and associates (1980) reported that in their study higher-ranking male long-tailed macaques performed less than lower-ranking males, and suggested that the social pressure of maintaining a high dominance rank resulted in poor performance. Furthermore, Nash and Chamove (1981) observed that high-ranking stump-tailed macaques (*Macaca arctoides*) "were more disturbed when removed from the group" (p. 88) than other animals.

Our results merit an expansion of these suggestions. We suggest that when removed from the group, the concerns involved in maintaining a high dominance rank lead animals to direct a larger amount of attention towards the group rather than to the test. Clearly, this can influence the results of a cognitive test.

A post-hoc analysis of our data provided a hint of a behavioural mechanism behind these results. For the nine subjects of the R-group we analysed the responses to trials after a total of 287 'disturbances', ranging between 24 to 38 disturbances per subject (taken irrespective of the type of reversal). These disturbances were accidental loud noises that could affect the performance of subjects, for instance, fights and manipulations of the cage by other group members (e.g. by forcefully hitting the wooden sliding doors). For each subject, we calculated the mean percentage errors made after disturbances and compared it with the mean percentage of errors made on an equal number of control trials, which were not preceded by a disturbance. The difference between these percentages was positive for all subjects, that is all subjects made more mistakes after such a disturbance. Furthermore, the results of a Pearson correlation between this difference and the dominance status of the subjects suggests that this relation is most pronounced in high-ranking subjects ($r = -0.66$, $N = 9$, $p = 0.051$), see Figure 3.6. This suggests that events in the group may be instrumental in diverting the attention of dominant animals away from the test. Future work could clarify this with detailed studies of the chronological relations between events in the group, attention and performance.

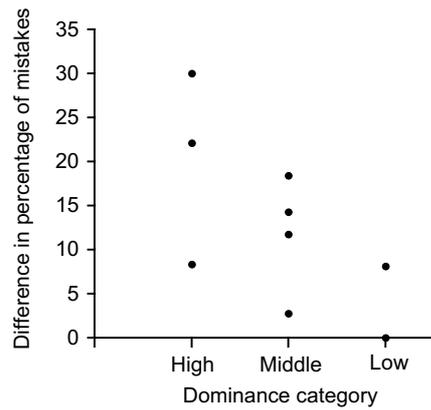


Figure 3.6. Difference in percentage of mistakes after a disturbance compared to control for the subjects of the R-group

Arousal

It was hypothesised that increased behavioural arousal would be associated with a reduction in performance. Additionally, we expected that social dominance and a lack of experimental experience would be related to increased behavioural arousal.

In contrast to our expectations, there was no statistical relation between dominance status and arousal. This is surprising in view of previous suggestions concerning behaviour of dominant individuals when removed from their group (Nash & Chamove, 1981). The disturbance of dominant animals, suggested by Bunnell et al. (1980) and Nash and Chamove (1981), was here expressed in a reduced attention, but not in an increase of behavioural arousal.

One other finding concerning behavioural arousal stands out. Subjects with previous experience on a cognitive test showed more behavioural arousal, which is in contrast with our expectation. Yet, experienced subjects had an improved performance relative to naïve animals, which is in line with our expectation. These findings suggest that the behavioural arousal indicated positive, task related excitement, rather than anxiety related to the test procedures.

It is known that the relationship between arousal and exposure to glucocorticoids on the one hand, and cognitive functioning on the other, is not linear but rather an inverted U-shape. Limited levels of glucocorticoid exposure may stimulate cognitive functioning rather than impair it (Diamond et al., 1992; Lupien & McEwen, 1997; Fuchs et al., 2001). Also, it is clear that behavioural arousal may indicate different underlying emotional states: anxiety and excitement (Lang, 1995; Thayer, 1996). Thus, the higher behavioural arousal of the experienced animals relative to the naïve subjects may be the result of their excitement or positive arousal in anticipation of the task at hand (Spruijt et al., 2001). Such energetic arousal (Thayer, 1996) would be expected to be positively related to the level of attention (cf. Spruijt et al., 2001).

A post-hoc analysis indeed revealed that the level of attention was higher in animals with a higher level of arousal, when controlling for the effect of present dominance status. This effect was in the same direction in the two types of reversals (Abstract: $F(1, 10) = 10.71$, $p = 0.08$; Food: $F(1, 10) = 2.36$, $p > 0.1$).

Nevertheless, it remains remarkable that the experienced subjects were more (positively) aroused than the naïve ones, even after an extensive period of training in which both groups were familiarised with the procedure. If at all, one would expect naïve animals to be more (negatively) aroused by the procedures. Apparently, the exposure to a similar test paradigm four years earlier gave the experienced subjects a substantial benefit in dealing with the procedures compared to the naïve animals. This advantage was expressed in an improved performance and higher level of excitement or anticipation.

Although some of our analytic models showed an effect of behavioural arousal on performance, further analysis revealed that these effects were the result of a co-linearity between performance and arousal. In GLMs controlling for the influence of experience on performance, behavioural arousal did not have an additional effect on performance. So, our data do not provide a clear answer to whether the positive effect of experience on performance is directly linked to an improved cognitive performance of the experienced subjects. It is also possible that the experienced subjects are more aroused or excited in relation to the task, which resulted in a better performance. Future studies applying a more detailed analysis of arousal (see Leavens et al., 2001 for an example) and when these behaviours occur during trials could determine the relative influence of the negative (anxiety) and positive (excitement) arousal in individuals.

Temperament & Biological Life Events

Obviously, the model presented here can be expanded further with additional factors affecting cognitive performance. Two obvious factors that have not been included in the present paper are temperamental differences between subjects and 'biological life events'.

Temperamental differences between animals (Capitanio, 1999; Gosling, 2001) may be important for understanding individual differences in behaviour and performance on cognitive tests (Schneider et al., 1991; Stavisky et al., 2000), as they are in humans (Eysenck, 1985; Arbuckle et al., 1986; Eysenck, 1997; Persson et al., 2000). Although a study examining a temperament trait of the present subjects has been conducted, this was not included here for statistical reasons.

In the human literature the combined number of certain events occurring during life, such as surgical interventions under general anesthesia, have been shown to constitute a risk factor for cognitive functioning at old age (Jolles et al., 1993). Yet, Dijkstra and co-workers (1998) did not find a separate effect of the number of such surgical interventions on age-related cognitive decline. In (captive) primates such 'biological life events' also occur, with the number of interventions under general anesthesia as a clear example. In our small sample it

was not possible to examine the relation between the number of such interventions and cognitive decline, because for our subjects this number correlated with their age. A study with a larger sample size, and especially more old subjects, should make it possible to separate these variables.

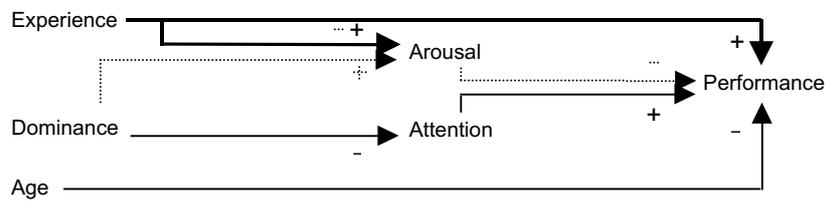


Figure 3.7. Representation of the authors' concluding interpretation of all the found relations between the factors studied here. Dotted arrows and signs (+/-) indicate expected relations between factors for which no conclusive evidence was found. The weight of the solid arrows indicates the strength of the relation between factors. A '+' indicates a positive relation: a high level in one factor is related to a high level in the other. A '-' indicates a negative relation: a high level in one factor is related to a low level in the other. A high level of performance means that few trials to criterion were needed and a low percentage of perseverative errors was made.

CONCLUSION

This study dealt with previous experimental experience, present dominance status, level of attention and arousal as factors that need to be considered in studies of cognitive capacities in socially housed monkeys. Additionally, this work examined how these factors are related to the age of subjects.

The level of attention, as an indication of motivation, was positively related to the performance of subjects, and negatively to their present dominance rank. We suggest that concerns involved in maintaining a high dominance rank when removed from the group led dominants to direct a larger amount of attention towards the group rather than to the test. Clearly, this can influence the results of a cognitive test. Our results also suggest that the arousal exhibited by the subjects was based on task-related excitement rather than on procedure-related anxiety.

In Figure 3.7 we give our interpretation of the relations between the factors studied here and general cognitive performance in our subjects. In sum, this study shows how attention and arousal influence cognitive performance of individual monkeys. Additionally, it gives indications of how these factors are in turn related to the social status and previous experience of individuals. Therefore, we conclude that the social environment is as important to consider in primate studies of cognition as it is in human studies.

ACKNOWLEDGEMENTS

We thank Michelle Doumen, Patricia Verschoor and Leonie de Visser for assisting in the data collection. Henk Westland and Annet Louwerse provided practical assistance. Han de Vries gave expert statistical advice.

APPENDIX

In this appendix a full account of the different steps taken in the analysis, and the results is given. For more information about the way the analysis was designed, see the analysis section of this chapter.

In this study the relation between experience, dominance status and differences in the amount of attention, arousal and individual cognitive performance was examined. For a summary of the data see Table 3.1; Figure 3.8 provides a summary of the relations we have found.

We first report the effect of experience, dominance rank category and age on the number of trials for the reversals. Also, we report the effect of these variables on the percentage of perseverative errors. Next, we describe how experience, age and present dominance status of the subjects affect the level of attention or arousal. Finally, we describe our findings with regard to the influence of attention and arousal on the number of trials on the reversals; and on the percentage of perseverative errors. We analysed 'abstract' and 'food' reversals separately. These findings were controlled for effects revealed in the first analysis.

A Effects of experience, dominance and age on performance measures

We analysed whether experience, age and the present dominance status affected the performance of our subjects. On both abstract and food reversals, experienced subjects required significantly fewer responses to reach the learning criterion (i.e. had a better performance) than naïve subjects (Abstract: $F(1, 9) = 29.92$, $p < 0.001$; Food: $F(1, 9) = 11.64$, $p = 0.008$). Age had a significantly positive effect on the number of trials on abstract reversals; the food reversals tended to show the same age-related impairment, even though this was not significant (Abstract: $F(1, 9) = 7.39$, $p = 0.024$; Food: $F(1, 9) = 4.10$, $p = 0.074$). Present dominance status was not significantly related to the number of trials in either type of reversals (Abstract: $F(2, 9) = 3.47$, $p = 0.076$; Food: $F(2, 9) = 0.20$, $p > 0.1$).

With respect to the percentage of perseverative errors, a non-significant interaction between experience and age was found for the abstract reversals (Experience-Age $F(1, 8) = 3.74$, $p = 0.089$). Additionally, a significant interaction between experience and dominance status was found, but there was no age effect (Experience-Dominance: $F(2, 7) = 7.38$, $p = 0.019$; Age: $F(1, 7) = 0.09$, $p > 0.1$). Further inspection showed that this significant interaction effect was primarily due to a difference between experienced and naïve animals in the middle dominance category (data not shown). When the interaction effect was omitted from the model, only experience had a significant influence on the perseverative tenden-

cies (Experience: $F(1, 9) = 6.41, p = 0.032$; Dominance: $F(2, 9) = 0.09, p > 0.1$; Age: $F(1, 9) = 0.01, p > 0.1$), with experienced subjects making fewer perseverative mistakes. For the food reversals no significant effects were revealed (Experience: $F(1, 9) = 4.86, p = 0.055$; Dominance: $F(2, 9) = 1.28, p > 0.1$; Age: $F(1, 9) = 0.53, p > 0.1$).

Thus, the number of trials decreased significantly with experience and increased with age for both types of stimuli (see above). The non-significant effect of age on the number of trials on the food reversals is in the same direction as the significant effect on the abstract reversals, therefore, we will also consider age in our further analysis of the food reversals (see below). Because dominance did not have a significant effect on the number of trials for either type of reversals, dominance will not be considered. We will correct the models concerning the effects of the behavioural measures on the perseverative errors with both experience and dominance.

B Relations with behavioural measures

The findings concerning the level of attention will be reported, followed by the results relating to the behavioural arousal of the subjects.

Level of attention

We first examined the relation between the level of attention and the performance measures for the abstract reversals, and then for the food reversals.

Abstract Reversals

I Effects of experience, dominance and age on the level of attention

We first examined how experience, dominance status and age influenced the level of attention. We performed a GLM with the level of attention as dependent variable with experience and dominance status as fixed factors; age was included as a co-variable. This analysis revealed that the highest ranking subjects showed the lowest level of attention ($F(2, 9) = 6.90, p = 0.015$; Figure 3.2A). Neither experience nor age further affected the level of attention (Experience: $F(1, 9) = 3.22, p > 0.1$; Age: $F(1, 9) = 0.10, p > 0.1$).

II Effects of the level of attention on the number of trials

A GLM with the trials to criterion as dependent measure and the level of attention as co-variable revealed that subjects with a high average level of attention needed significantly fewer trials to reach criterion on abstract reversals than subjects with a low level of attention ($F(1, 12) = 10.38, p = 0.007$).

In the following model we incorporated experience and age to investigate whether the level of attention had an additional effect on the number of trials over and above the effect of these variables. This yielded one significant interaction effect (Experience-Age: $F(1, 9) = 5.56, p = 0.043$) and one that approached significance (Experience-Attention: $F(1, 9) = 5.03, p = 0.052$). This suggests that the effects of age and the level of attention were not as strong in the naïve subjects as they were in the experienced subjects. (Figure 3.2B). Furthermore, in the model with the significant interaction effect between experience and

age, there was also a significant effect of attention ($F(1, 9) = 19.59, p = 0.002$). Thus, the level of attention did have an additional positive effect on performance.

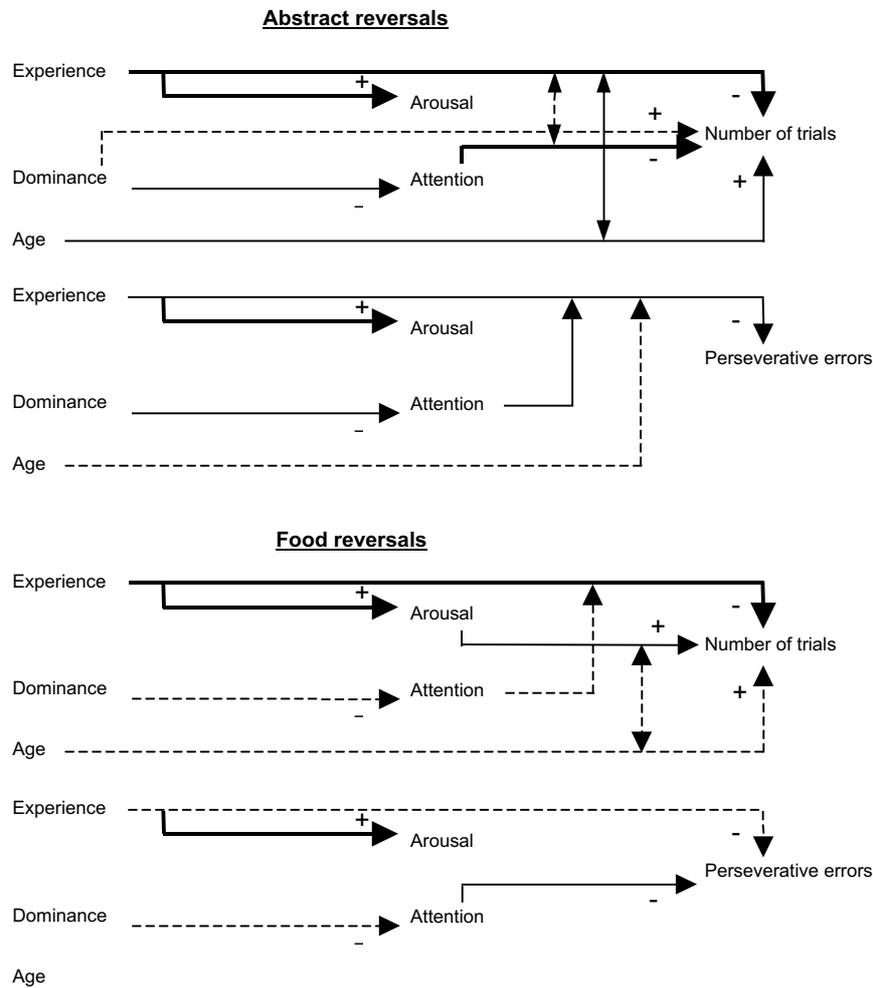


Figure 3.8. Representation of the found relations between the factors studied here. Striped arrows signify relations with $p \leq 0.10$; solid arrows: $p \leq 0.05$; bold arrows: $p \leq 0.01$. Arrows pointing at other arrows indicate interaction effects.

A '+' indicates a positive relation: a high level in one factor is related to a high level in the other.

A '-' indicates a negative relation: a high level in one factor is related to a low level in the other.

For the number of trials a high level means that many trials to criterion were needed. For perseverative errors a high level means a high percentage of perseverative errors was made.

III Effects of the level of attention on the perseverative errors

A GLM with the percentage of perseverative errors as dependent variable and the level of attention as co-variable provided no significant result ($F(1, 12) = 0.16, p > 0.1$).

In the next step, we added experience and dominance status as fixed factors to this model. Earlier, we reported that a significant interaction effect between experience and dominance status affects the percentage of perseverative errors (see above). In addition, we now found a non-significant interaction between dominance status and attention ($F(2, 7) = 3.64, p = 0.082$). Finally, there was a significant interaction between experience and the level of attention ($F(1, 8) = 5.35, p = 0.050$). Experienced subjects appear to make fewer perseverative errors when they show more attention, while this is not true for naïve subjects (Figure 3.2C).

Food Reversals

I Effects of experience, dominance and age on the level of attention

We first examined how experience, dominance status and age influenced the level of attention. We performed a GLM with the level of attention as dependent variable and experience and dominance status as fixed factors; age was included as a co-variable. The significant effect of dominance status on the level of attention for the abstract reversals did not reach significance for the food reversals ($F(2, 9) = 3.09, p = 0.095$; Figure 3.3A), although the trend was in the same direction. Neither experience nor age further affected the level of attention (Experience: $F(1, 9) = 0.87, p > 0.1$; Age: $F(1, 9) = 1.14, p > 0.1$).

II Effects of the level of attention on the number of trials

The relation between the level of attention and the number of trials that was reported for the abstract reversals was not evident for the food reversals ($F(1, 12) = 0.24, p > 0.1$).

In the following GLM we incorporated experience and age in the model to investigate whether the level of attention had an additional effect over and above the effect of these variables. The interaction effect between experience and the level of attention found for the abstract reversals was not significant here ($F(1, 9) = 4.23, p = 0.070$; Figure 3.3B). The model with only the main effect showed that the level of attention had no additional effect on the number of trials besides experience and age (Experience: $F(1, 10) = 11.53, p = 0.007$; Age: $F(1, 10) = 5.95, p = 0.035$; Attention: $F(1, 10) = 0.01, p > 0.1$).

III Effects of the level of attention on the perseverative errors

Subjects with a high average level of attention made significantly fewer perseverative errors on food reversals than subjects with a low level of attention ($F(1, 12) = 5.04, p = 0.044$; Figure 3.3C).

Although no significant effect of either experience, dominance or age was obtained for the perseverative errors on food reversals (see above), we did test a GLM with the perseverative errors as dependent variable with experience and dominance as fixed factors, and the level of attention as co-variable. This analysis, which had resulted in interaction effects for the abstract reversals, did not yield any significant results for the food

reversals (Experience: $F(1, 9) = 3.55, p = 0.092$; Dominance: $F(2, 9) = 0.46, p > 0.1$; Attention: $F(1, 9) = 2.12, p > 0.1$).

Arousal

The second behavioural variable under examination in this paper is arousal, measured as the rate of displacement behaviour. We first analysed how the experience, dominance status and age of the subjects affect this behavioural measure of arousal. Then we investigated how it is associated with the performance of the subjects. Again, these analyses were done separately for the abstract and food reversals.

Abstract Reversals

I Effects of experience, dominance and age on arousal

We first examined whether experience, dominance status and age influenced arousal. We performed a GLM with arousal as dependent variable with experience and dominance status as fixed factors; age was included as a co-variable. Experienced animals had a higher arousal than naïve subjects did (Experience: $F(1, 9) = 13.48, p = 0.005$). There was no effect of either dominance status or age (Dominance: $F(2, 9) = 1.40, p > 0.1$; Age: $F(1, 9) = 0.01, p > 0.1$; Figure 3.4A).

II Effects of arousal on the number of trials

Subjects with high level of arousal needed fewer trials to reach criterion on abstract reversals than subjects with a low level of arousal; this effect approached significance ($F(1, 12) = 4.53, p = 0.055$).

In the following GLM we incorporated experience and age in the model with the number of trials as dependent variable to investigate whether the level of arousal had an additional effect over and above the effect of these variables. No additional effect of arousal was found (Experience: $F(1, 10) = 5.41, p = 0.042$; Age: $F(1, 10) = 11.90, p = 0.006$; Arousal: $F(1, 10) = 0.96, p > 0.1$; Figure 3.4B). So, experience was related to both a low number of trials and a high level of arousal, but the level of arousal was not directly related with the number of trials.

III Effects of arousal on the perseverative errors

A GLM with the percentage of perseverative errors as the dependent variable and arousal as co-variable provided no significant result ($F(1, 12) = 3.93, p = 0.071$).

In the next step, we added experience and dominance status as fixed factors to this model. Again, arousal did not add an effect to the interaction effect of experience and dominance status reported above (Experience-Dominance: $F(2, 7) = 7.26, p = 0.020$; Arousal: $F(1, 7) = 0.05, p > 0.1$; Figure 3.4C). So, experience was related to both a low perseverative tendency and a high level of arousal, but arousal was not directly related with perseveration.

Food Reversals

I Effects of experience, dominance and age on arousal

We first examined whether experience, dominance status and age influenced arousal. We performed a GLM with arousal as dependent variable with experience and dominance status as fixed factors; age was included as a co-variable. This analysis, again, showed that experienced animals had a higher arousal than naïve subjects did (Experience: $F(1, 9) = 10.41, p = 0.010$; Dominance: $F(2, 9) = 1.30, p > 0.1$; Age: $F(1, 9) = 0.45, p > 0.1$; Figure 3.5A).

II Effects of arousal on the number of trials

Subjects with a high arousal needed fewer trials to reach criterion on food reversals than subjects with a low arousal did ($F(1, 12) = 5.23, p = 0.041$).

In the following GLM we incorporated experience and age in the model with the number of trials as dependent variable to investigate whether the level of arousal had an additional effect over and above the effect of these variables. We found an interaction effect between age and arousal with a p-value of 0.077 ($F(1, 9) = 4.00$). When this interaction was omitted from the model, arousal did not have an additional effect (Experience: $F(1, 10) = 4.06, p = 0.072$; Age: $F(1, 10) = 6.45, p = 0.029$; Arousal: $F(1, 10) = 0.76, p > 0.1$). Experience was related to both a low number of trials and a high level of arousal, but arousal was not directly related with performance (Figure 3.5B). This is the same as in the abstract reversals.

III Effects of arousal on the perseverative errors

A GLM with the percentage of perseverative errors as dependent variable and arousal as co-variable provided no significant result ($F(1, 12) = 0.46, p > 0.1$).

Although no significant effect of either experience, dominance or age were obtained for the perseverative errors on food reversals (see above), we did test a GLM with the perseverative errors as dependent variable with experience and dominance as fixed factors, and arousal as co-variable. This model did not provide any significant results (Experience $F(1, 9) = 4.20, p = 0.071$; Dominance: $F(2, 7) = 0.80, p > 0.1$; Arousal: $F(1, 7) = 0.47, p > 0.1$; Figure 3.5C).

CHAPTER 4

Effects of trait anxiety on performance and behaviour of socially housed monkeys in a learning test

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ABSTRACT

Studies of individual cognitive capacities of socially housed monkeys may profit from a better understanding of the factors that influence individual behaviour and performance in test procedures. Trait anxiety of subjects may be an essential factor affecting their reaction to, and performance in cognitive tests. This study related trait anxiety of 14 socially housed monkeys with their performance and behaviour in an individual Discrimination-Reversal learning test. Results suggest that high trait anxiety reduces the willingness to co-operate in test procedures, attention for the task, and cognitive performance. Test-related excitement was reduced in high trait anxiety naïve subjects, but not in subjects with previous experimental experience. Conceptual and methodological limitations of this study are discussed.

INTRODUCTION

Testing individual cognitive capacities of socially housed animals is important for understanding the processes involved in the well known phenomenon of age-related cognitive decline. However, as previous work in our laboratory (see previous Chapters) has shown, no robust method has yet been found. Sample sizes were small due to a time-consuming training period, and inter-individual variation was large. Individual differences in temperament of primates (for a review see Gosling, 2001; Itoh, 2002) may be related to this inter-individual variation (Boccia et al., 1995; Watson & Ward, 1996; Stavisky et al., 2000; Ward et al., 2003). Individual differences in trait anxiety or fearfulness constitute an important aspect of primate temperament (Higley & Suomi, 1989; Suomi, 1991b; Bolig et al., 1992; Clarke & Boinski, 1995; Gosling, 2001), even more so, considering the suggested interconnectedness of anxiety and cognition (McNaughton, 1997). This paper will address the explanatory value of individual differences in anxiety of monkeys in determining their cognitive performance and behavioural responses to test procedures. Additionally, this study will add to scarce knowledge of the relation between individual differences in trait anxiety and cognitive performance in primates.

Non-human primates have been reported to show individual differences in trait anxiety (Schneider et al., 1991; Boissy, 1995). Individuals have stable ways of reacting to fear- or anxiety-evoking situations (Higley & Suomi, 1989; Suomi et al., 1996; Bowers et al., 1998), often referred to as 'trait anxiety' (Eysenck, 1997). Clearly, individual differences in temperament aspects, such as trait anxiety, are dependent on both genetic influences and ontology (Suomi, 1987; Clarke & Boinski, 1995; Bouchard & McGue, 2003; Finn et al., 2003; Fairbanks et al., 2004). A social environment, i.e. a natural social group, is a crucial aspect of ontology, as it is essential for normal cognitive and emotional development of primates (Suomi, 1991a; Sanchez et al., 1998; Sanchez et al., 2001; Lutz et al., 2003). This study will therefore investigate trait anxiety and cognitive performance in socially housed monkeys.

Differences in trait anxiety have been shown to limit cognitive performance in rodents (Ohl et al., 2002; Ohl et al., 2003), humans (Eysenck, 1985; Revelle, 1987; Mueller, 1992; Kumari & Corr, 1998; Persson et al., 2000; Li et al., 2004) and primates (Schneider et al., 1991). Furthermore, studies of cognitive capacities of primates may require procedures that could evoke anxiety in experimental animals. For instance, Itoh (2001) reported that both rhesus (*Macaca mulatta*) and Japanese monkeys (*M. fuscata*) differ in their individual tendency to approach an unknown human feeder. Additionally, Higley and Suomi (1989) reported that rhesus monkeys show individual differences in their reaction to temporary isolation.

The experimental procedures of a study reported previously (Chapter 2 and 3) contained similar elements. In that study, concerning individual cognitive capacities of socially housed long-tailed macaques (*Macaca fascicularis*), subjects had to approach a human experimenter up to a distance of approximately 50 centimetres in order to enter a learning compartment. They would remain in the learning compartment, physically isolated from the group for the duration of a test session. These procedures may have led to individual differences in anxiety or fear during the cognitive test, and thus to individual differences in performance.

In the present study we aim to evoke anxiety in the same subjects who had previously participated in the cognitive test (Chapter 2 and 3). We expect that individual differences in anxiety obtained in the present study will relate to individual differences in anxiety in the cognitive test procedures, as well as to differences in behaviour and cognitive performance.

Previously, we have shown that the behavioural arousal of the subjects during the cognitive test was associated with both improved performance and previous experimental experience (Chapter 3). We proposed that the behavioural arousal of the subjects should therefore be interpreted as indicating task related excitement. Individual differences in trait anxiety would then be expected to reduce behavioural arousal.

Most studies investigated individual differences in anxiety by presenting either solitary or pair housed monkeys with a challenging situation such as novel objects or environments (Crepeau & Newman, 1991; Vitale et al., 1991; Vochteloo et al., 1991; Watson & Ward, 1996; Joly et al., 2001). For our study the use of novel objects as challenge was less suitable, because the reactions of monkeys tested within the context of their social group could then be dependent on the social environment. For instance, subordinate monkeys may approach novel objects depending on the location and behaviour of more dominant individuals. Furthermore, not all group members may notice a novel object at the same time. Rather, in this study we challenged groups of monkeys with a sudden, loud noise. All group members perceive a loud noise at the same moment. Also, a sound cannot be dominated like an object. So it allowed a more individually based response, than the more commonly used novel object. Thus, no effects of age or dominance status are expected. We will take the behavioural response of subjects as a measure of their trait anxiety.

In sum, we expect animals with high scores on trait anxiety to be less willing to enter the learning compartment for the cognitive test. Furthermore, we hypothesise that these animals show a poor performance in the cognitive test. Finally, we predict that high trait anxiety is related to reduced attention (Mueller, 1992), and reduced behavioural arousal in the cognitive test.

METHODS

Individual differences in trait anxiety

Subjects and housing

Two groups of socially housed long-tailed macaques (*Macaca fascicularis*) participated in this study, the R-, and the L-group. Both groups were housed in large indoor (80 m², 3 m high) - outdoor (200 m², 4 m high) enclosures at the Ethology Station of Utrecht University. Both groups consisted of a number of adult males and females and their offspring. The total number of individuals in each group was 50 and 30, respectively at the start of testing. Fifteen female subjects were selected: nine from the R-group and six from the L-group. These were the subjects that had previously participated in a Discrimination-Reversal learning test (see Chapter 2). Groups were fed twice a day (Hope Farms 2500 Apenvoeder G.O. 14 mm, monkey chow), with additional fruit and vegetable being offered twice a week. Water was available ad libitum.

The two groups were housed in adjacent enclosures, one of which was equipped with the noise producing devices described below. After testing the R-group, the groups switched enclosures, so that the L-group could be tested. Both groups were housed in the test enclosure at least three weeks before testing began.

Experimental Set-up

This experiment was aimed at obtaining a measure of trait anxiety for each subject. To that end both groups were repeatedly exposed to a sudden loud noise.

A commercially available air-horn was used to produce this noise (114 dB, measured directly underneath the horn at a height of 1.5 m). The horn was placed above the middle of the indoor enclosure (see Figure 4.1, e), it was connected with an air canister outside the enclosure by a flexible, plastic tube. Also, above the middle of the enclosure were two speakers, which were connected to an audio-cassette player outside the cage. This was used to offer two other sounds, one of which was always presented just prior to the noise. These were neutral sounds in the sense that they did not generate any response when they were tested on a third group of monkeys, in contrast to the air-horn noise. The interval between this neutral sound and the air-horn noise ranged between six seconds on the first six presentations and 66 seconds on the latter six presentations. The groups were exposed to the noise on twelve occasions over a period of four weeks. Originally, this study aimed to elicit a conditioned fear response, however, a preliminary analysis of the data from the R-group revealed no signs of such a response (data not shown). Furthermore, no habituation effect of the twelve presentations was found for either group (data not shown). Therefore, this study will focus on the

behaviour of subjects in response to the noise, averaged over twelve presentations.

The noise was administered three times on each of four possible times of day (10:00, 11:00, 14:00 or 15:00 hours) in a pseudo-random order. On test days the monkeys were locked in their indoor enclosure at 9:00 hours. Behaviour of the monkeys was videotaped using two video cameras (Figure 4.1) from fifteen minutes prior (control observations, see below) to fifteen minutes after the noise. These video recordings were later used to observe the behaviour of each of the

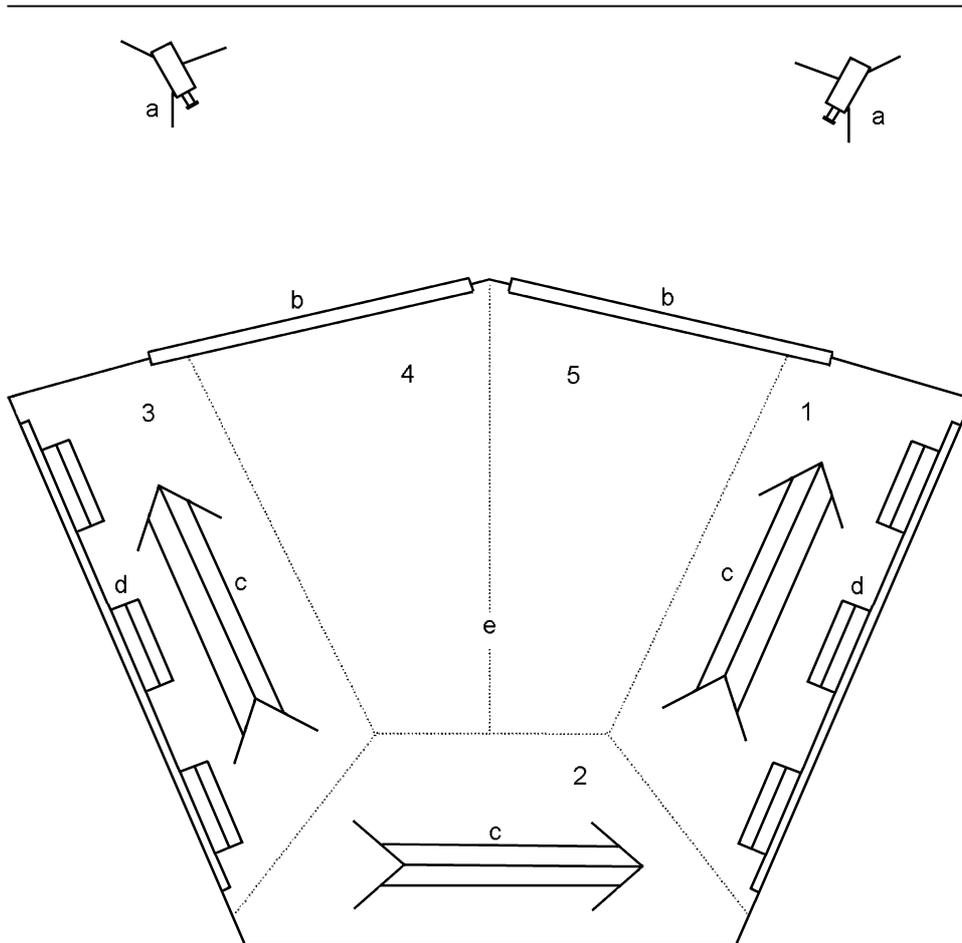


Figure 4.1. Schematic top-view of the inside homecage, where the acoustic stimulus was presented (a: videocamera; b: observation windows; c: scaffolding construction; d: wall-rack to facilitate climbing & additional sitting platforms; e: location of stimulus generator; 1-5: imaginary compartments, divided by dotted lines).

subjects in response to the noise. Two observers, both trained by IT, observed the videotapes of the two groups. Observations included behaviours indicating (in)activity (such as walking, running, climbing and sitting); arousal (such as scratching, body shaking); grooming and self-grooming (cf. Angst, 1974; De Waal et al., 1976; Schino et al., 1996).

Measures of trait anxiety

The behavioural reaction of subjects to the noise was quantified using six measures, indicating: 1) the distance subjects fled in reaction to the noise; 2) the latency to leave the location the subject fled to; 3) the rate of behavioural changes; 4) the rate of scratching; 5) the relative duration of active behaviour; 6) the latency to the first grooming bout. These measures were corrected for control values derived from the observations prior to the noise. For a more detailed description see the appendix at the end of this chapter.

A factor analysis with an orthogonal (varimax) rotation was used to extract principal factors from these six variables quantifying the reaction of the subjects. This resulted in three factors, which together explained 88 percent of the total variation in the six variables. See Table 4.1 for the factor loadings. These three factors will henceforth be labelled as: *relax latency*, *activity*, and *distance*.

Table 4.1. Factor loadings for three factors of trait anxiety.

| Variable | Trait anxiety factors | | |
|---------------------------------|-----------------------|----------|----------|
| | Relax latency | Activity | Distance |
| LOG Latency leave flee location | 0.973 | | |
| Latency self-grooming | 0.751 | | 0.364 |
| Behavioural changes | 0.122 | 0.947 | -0.228 |
| Active behaviour | -0.154 | 0.893 | 0.369 |
| Distance | | 0.113 | 0.974 |
| Scratching | -0.553 | 0.121 | -0.673 |

Discrimination-Reversal test

The cognitive task was a Discrimination-Reversal (DR) learning test in which subjects were required to choose one stimulus out of an array of four stimuli. Subjects had to learn which stimulus was consistently associated with a reward (for more details on this test, see Chapter 2). When this initial discrimination had been learned, and each subsequent time a subject reached the pre-set learning criterion the association with the reward was switched (reversed) to one of the other stimuli. All subjects but one performed more than one of these reversals. Subjects received a maximum of 50 trials per day and could use multiple days to reach the pre-set learning criterion. Six of the subjects of the R-group had prior experimental experience on a similar cognitive test.

Measures of performance and behaviour in the Discrimination-Reversal test

Four measures to indicate the performance and behaviour of subjects on the reversal tasks of the DR-test were derived from Chapter 3. These measures comprised two variables concerning the cognitive performance of subjects, namely the *number of trials to criterion*, and the *percentage of perseverative errors*. Two others describe aspects of the behaviour of the subjects in the DR-test, namely the *level of attention* for the task, and the level of behavioural *arousal*. See Chapter 3 for additional information concerning these four measures. In addition a measure indicating the willingness of the subjects to enter the learning compartment was determined. Subjects co-operated on the DR-test voluntarily. The order in which animals entered the learning compartment of the experimental cage (see Figure 2.1) was logged on each test day. An average *test order* was calculated for each subject.

Analysis

First, we examined the results of the factor analysis. We tested if the resultant factors were related to the present dominance status of the subjects and their age.

Then we used the resultant factors to test our expectations. The DR-test involved four stimuli of two kinds, two 'abstract' stimuli and two 'food' stimuli (for more information see Chapters 2 and 5). Reversals on which an abstract- or food stimulus was correct were analysed separately, for statistical reasons. These separate analyses were done using the same scheme of subsequent General Linear Models (GLMs). We tested the effects on four independent variables: trials to criterion, percentage of perseverative errors, level of attention and arousal. For all dependent variables we first tested whether the trait anxiety factors alone had a significant effect. Next, we incorporated factors which have previously been shown to affect these dependent variables (Chapter 2 and 3), for instance the age and previous experience of our subjects. In these GLMs we included dyadic interaction terms in separate runs of the model; only interactions with a p-value less than 0.10 are reported here (see the analysis section of Chapter 3 for some additional notes on interaction terms). Due to statistical limitations not all factors under study here could be incorporated simultaneously in the models.

A large number of statistical tests were done, for both the abstract and food reversals. Most of the effects found reached significance for either abstract or food reversals. However, the non-significant relations were always in the same direction as the significant effect. In our report and discussion of the results, we will, for reasons of clarity and brevity, mention significant findings irrespective of whether these were found for the abstract or food reversals. In Tables 4.4-4.7 and Figures 4.3-4.5 we do provide results for both types of reversals. Also, effects of factors previously shown to affect performance and behaviour in the DR-test (Chapter 2 and 3), such as age, are not explicitly reported here.

I Examination of factors of trait anxiety

We tested whether the resultant factors of trait anxiety from the factor analysis were associated with dominance status and age of the subjects. The trait anxiety factors were separately analysed in General Linear Models with dominance status as fixed factor and age as co-variable.

II Effect of trait anxiety on test order of the DR-test

Subjects participated voluntarily in the DR-test. The experimenter did not determine the order in which subjects entered the test compartment (see Figure 2.1). Individual differences in trait anxiety may affect the willingness of subjects to enter this learning compartment. Therefore, we tested whether the anxiety factors affected the test order of the DR-test.

First, we tested whether the test order depended on experience, present dominance status or age of the subjects. Next, we examined whether the factors of trait anxiety alone influenced performance. Then we added the significant factors from the former GLM.

III Effect of trait anxiety on performance in the DR-test

We examined whether trait anxiety factors explained part of the variation found in the performance on the DR-test, in addition to the factors tested in Chapter 2. First, we tested whether the factors of trait anxiety alone influenced performance. Then we added the factors which had previously (Chapter 2) proven to affect performance.

IV Effect of trait anxiety on behaviour in the DR-test

We investigated whether the trait anxiety factors affected the behaviour of the subjects while they co-operated on the DR-test, in addition to the factors tested in Chapter 3. First, we tested whether the factors of trait anxiety alone influenced performance. Then we added the factors which had previously (Chapter 3) proven to affect behaviour.

RESULTS

The aim of the present work was to relate individual differences in trait anxiety of monkeys to behaviour and performance of these monkeys in a Discrimination-Reversal (DR) learning test.

In this section only the most relevant relations are presented for reasons of clarity. The appendix at the end of this chapter provides a full account of the results.

I Examination of factors of trait anxiety

The first factor is mainly determined by the latency to leave a flee location and the latency to the first bout of self-grooming. Both these variables can be seen as indicating the moment at which animals recover from a period of anxiety (Higley & Suomi, 1989; Aureli & Van Schaik, 1991; Spruijt et al., 1992), in other words a *relax latency*.

The main factor loadings of second trait anxiety factor come from the rate of behavioural changes and the relative duration of activity. Thus, this factor can be seen as signifying the level of activity in the fifteen minutes after noise onset (Crepeau & Newman, 1991; Capitanio, 1999; Kaplan et al., 2001; Aguilar et al., 2002). We shall call this factor *activity*.

The third trait anxiety factor, labelled 'distance', is primarily determined by the distance covered by the subjects after the onset of the noise and, inversely, by the scratching rate. This relation of a greater distance and a reduced scratching rate over fifteen minutes of observation makes this factor more difficult to interpret in the sense of trait anxiety. In view of this interpretation difficulty, and because the goal of this paper is to test hypotheses concerning the relation between trait anxiety and cognitive performance, we will focus on the effects of the first two trait anxiety factors.

So, two factors were further used to investigate the relation between individual trait anxiety and performance and behaviour in the DR-test. These factors were not associated with either present dominance status or age of the subjects (Table 4.2).

Table 4.2. Effects of dominance and age on trait anxiety factors

| | D.F. | Trait anxiety factors | | | |
|-----------|---------|-----------------------|---------|----------|---------|
| | | Relax latency | | Activity | |
| | | F-Value | P-Value | F-Value | P-Value |
| Dominance | (2, 11) | 0.74 | p > 0.1 | 0.67 | p > 0.1 |
| Age | (1, 11) | 0.01 | p > 0.1 | 0.01 | p > 0.1 |

Only models with interactions with a P-value of less than 0.10 are reported, if no such interactions were found on either of the types of reversals, only the main effects of the models are shown.

II Effect of trait anxiety on test order of the DR-test

For the factor relax latency two interaction effect were found: between experience and relax latency, and between dominance status and experience (Table 4.3). These interactions complicate the interpretation of the relation between the factor relax latency and test order. However, subjects with a high level of the factor activity were less willing to enter the learning compartment than other subjects (Table 4.3, Figure 4.2) when controlling for previous experience

and dominance status. This shows that two indications of individual differences in anxiety are associated.

Table 4.3. Effect of trait anxiety factors on test order of the DR-test

| | D.F. | F-Value | P-Value | |
|---------------------------------------|--------------------------|----------|----------------|--------------|
| <u>Experience-Dominance-Age</u> | | | | |
| Subsequent General Linear Models ↓ | Experience | F(1, 10) | 0,011 + | |
| | Dominance | F(2, 10) | > 0.1 | |
| | Age | F(1, 10) | > 0.1 | |
| | Experience*Dominance | F(2, 9) | 0,077 | |
| | Experience | F(1, 11) | 10,943 | |
| | Dominance | F(2, 11) | 1,039 | |
| | <u>Relax latency</u> | | | |
| | Relax latency | F(1, 13) | 0,03 | > 0.1 |
| | Dominance | F(2, 9) | 4,609 | 0,042 |
| | Experience*Relax latency | F(1, 9) | 12,62 | 0,006 |
| Relax latency | F(1, 8) | 2,989 | > 0.1 | |
| Experience*Dominance | F(2, 8) | 5,532 | 0,031 | |
| <u>Activity</u> | | | | |
| Activity | F(1, 13) | 0,66 | > 0.1 | |
| Activity | F(1, 8) | 5,315 | 0,05 + | |
| Experience*Dominance | F(2, 8) | 5,79 | 0,028 | |

Only models with interaction effects with a P-value of less than 0.10 are reported, when no such interaction effects were found on either of the types of reversals, only the main effects of the models are shown. P-values of less than 0.1 are indicated in bold.

III Effect of trait anxiety on performance in the DR-test

High levels of trait anxiety were expected to be related to a lower cognitive performance, i.e. to high numbers of trials to criterion. This expectation was confirmed for the factor activity. Subjects with high scores on the factor activity needed more trials to reach criterion than subjects with low scores (Table 4.4, Figure 4.3). For the factor relax latency only an interaction effect between experience and relax latency was found, which approached significance.

Also, high trait anxiety scores were expected to be related to a high percentage of perseverative errors. However, neither one of the trait anxiety factors

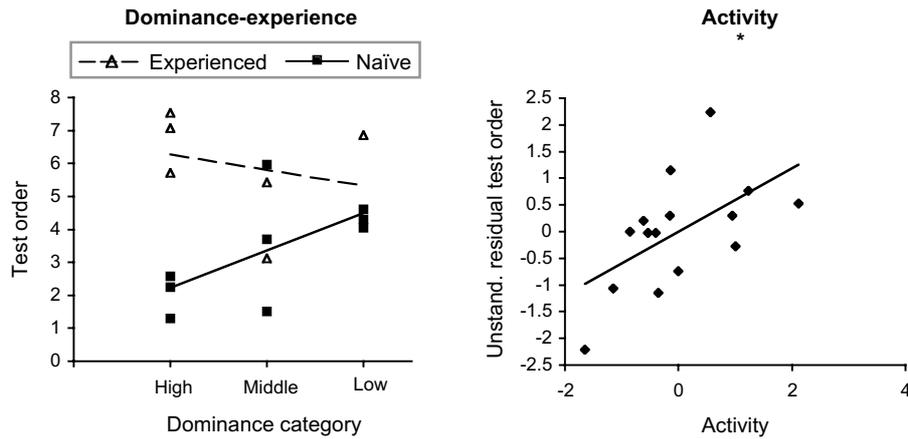


Figure 4.2. The effect of dominance category and experience on test order, and the effect of the trait anxiety factor activity on test order (* $p \leq 0.05$; ** $p \leq 0.01$). The residual values are corrected for the influence of previous experimental experience, present dominance status and their interaction.

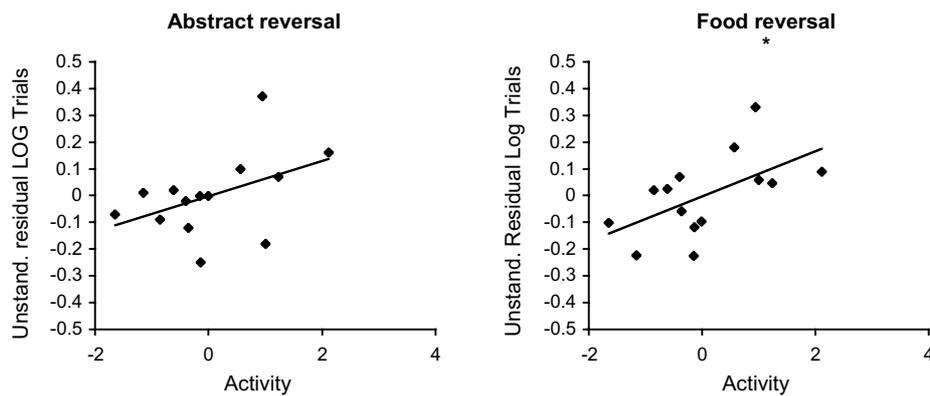


Figure 4.3. The relation between the trait anxiety factor activity and the LOG of the number of trials to criterion, for the abstract reversals and food reversals (* $p \leq 0.05$; ** $p \leq 0.01$). The residual values are corrected for the influence of previous experimental experience and age.

was significantly related to the perseverative tendency of the subjects (Table 4.5).

Still, these results provide support for the overall prediction that cognitive performance is negatively affected by trait anxiety.

Table 4.4. Effect of trait anxiety factors on trials to criterion in the DR-test

| | | D.F. | Abstract reversals | | Food reversals | |
|----------------------------------|--------------------------|----------|--------------------|--------------|----------------|----------------|
| | | | F-Value | P-Value | F-Value | P-Value |
| <u>Relax latency</u> | | | | | | |
| Subsequent General Linear Models | Relax latency | F(1, 12) | 0,18 | > 0.1 | 0,56 | > 0.1 |
| | Age | F(1, 9) | 6,892 | 0,028 + | 3,472 | 0,095 + |
| | Experience*Relax latency | F(1, 9) | 4,525 | 0,062 | 1,573 | > 0.1 |
| | Experience | F(1, 10) | 16,575 | 0,002 - | 10,494 | 0,009 - |
| | Age | F(1, 10) | 10,123 | 0,01 + | 6,017 | 0,034 + |
| | Relax latency | F(1, 10) | 0,103 | > 0.1 | 0,054 | > 0.1 |
| <u>Activity</u> | | | | | | |
| Subsequent General Linear Models | Activity | F(1, 12) | 1,9 | > 0.1 + | 3,47 | 0,087 + |
| | Experience | F(1, 10) | 19,986 | 0,001 - | 16,176 | 0,002 - |
| | Age | F(1, 10) | 10,736 | 0,008 + | 6,773 | 0,026 + |
| | Activity | F(1, 10) | 2,497 | > 0.1 + | 5,051 | 0,048 + |

Only models with interaction effects with a P-value of less than 0.10 are reported, when no such interaction effects were found on either of the types of reversals, only the main effects of the models are shown. P-values of less than 0.1 and concerning the trait anxiety factors are indicated in bold.

Table 4.5. Effect of trait anxiety factors on percentage of perseverative errors in the DR-test

| | | D.F. | Abstract reversals | | Food reversals | |
|----------------------------------|---------------|----------|--------------------|---------|----------------|---------|
| | | | F-Value | P-Value | F-Value | P-Value |
| <u>Relax latency</u> | | | | | | |
| Subsequent General Linear Models | Relax latency | F(1, 12) | 0,08 | > 0.1 | 0,09 | > 0.1 |
| | Experience | F(1, 11) | 9,013 | 0,012 - | 4,453 | 0,059 - |
| | Relax latency | F(1, 11) | 1,601 | > 0.1 | 0,955 | > 0.1 |
| <u>Activity</u> | | | | | | |
| Subsequent General Linear Models | Activity | F(1, 12) | 0,14 | > 0.1 | 0,37 | > 0.1 |
| | Experience | F(1, 11) | 7,1 | 0,022 - | 3,772 | 0,078 - |
| | Activity | F(1, 11) | 0,457 | > 0.1 | 0,693 | > 0.1 |

Only models with interaction effects with a P-value of less than 0.10 are reported, when no such interaction effects were found on either of the types of reversals, only the main effects of the models are shown. P-values of less than 0.1 and concerning the trait anxiety factors are indicated in bold.

IV Effect of trait anxiety on behaviour in the DR-test

In line with our predictions, subjects with high scores on the factor relax latency showed relatively low levels of attention for the task (Table 4.6, Figure 4.4). Yet, no effect of the factor activity was revealed.

Table 4.6. Effect of trait anxiety factors on attention in the DR-test

| | D.F. | Abstract reversals | | Food reversals | |
|----------------------|----------|--------------------|----------------|----------------|---------|
| | | F-Value | P-Value | F-Value | P-Value |
| <i>Relax Latency</i> | | | | | |
| Relax latency | F(1, 12) | 0,94 | > 0.1 | 0,27 | > 0.1 |
| <i>Dominance</i> | | | | | |
| Dominance | F(2, 10) | 15,687 | 0,001 - | 2,492 | > 0.1 |
| Relax latency | F(1, 10) | 10,656 | 0,009 - | 0,666 | > 0.1 - |
| <i>Activity</i> | | | | | |
| Activity | F(1, 12) | 0,84 | > 0.1 | 0,49 | > 0.1 |
| <i>Dominance</i> | | | | | |
| Dominance | F(2, 10) | 5,099 | 0,03 - | 1,905 | > 0.1 |
| Activity | F(1, 10) | 0,003 | > 0.1 | 0,004 | > 0.1 |

Only models with interaction effects with a P-value of less than 0.10 are reported, when no such interaction effects were found on either of the types of reversals, only the main effects of the models are shown. P-values of less than 0.1 and concerning the trait anxiety factors are indicated in bold.

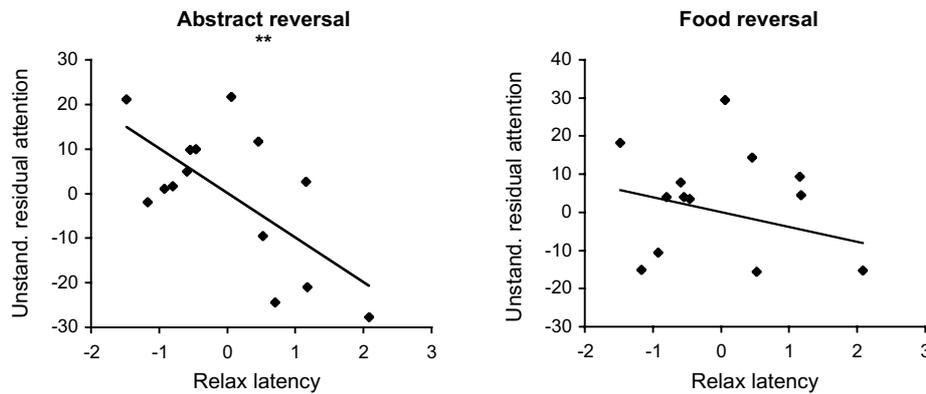


Figure 4.4. The relation between the trait anxiety factor relax latency and the attention, for the abstract reversals and food reversals (* $p \leq 0.05$; ** $p \leq 0.01$). The residual values are corrected for the influence of the present dominance status.

Furthermore, high levels of trait anxiety were expected to be related to reduced behavioural arousal during the DR-test. The initial model testing only the effect of the trait anxiety factors supported this prediction. Subjects with a high score on the factor relax latency showed less behavioural arousal. However, when previous experimental experience, which had previously been shown to affect the arousal (Chapter 3), was incorporated into the model it became clear that the relation between trait anxiety and arousal was more complex. An interaction effect between the factor relax latency and experience was found to influence behavioural arousal (Table 4.7, Figure 4.5). Further, post hoc analysis revealed that the effect for the experienced animals was not significant (Abstract: $F(1, 4) = 3.05, p > 0.1$; Food: $F(1, 4) = 0.33, p > 0.1$). However, for the naïve animals high scores on the factor relax latency were significantly related to reduced arousal (Abstract: $F(1, 6) = 5.16, p = 0.064$; Food: $F(1, 6) = 15.91, p = 0.007$). Again, the factor activity did not yield significant results.

So, these results indicate high trait anxiety reduced the level of attention for the task. Moreover, behavioural arousal was subdued in subjects with a high trait anxiety, but only in naïve subjects.

Table 4.7. Effect of trait anxiety factors on arousal in the DR-test

| | | D.F. | Abstract reversals | | Food reversals | |
|----------------------------------|--------------------------|----------|--------------------|--------------|----------------|--------------|
| | | | F-Value | P-Value | F-Value | P-Value |
| Subsequent General Linear Models | <u>Relax latency</u> | | | | | |
| | Relax latency | F(1, 12) | 2,29 | > 0.1 - | 5,31 | 0,04 - |
| | Experience*Relax latency | F(1, 10) | 7,019 | 0,024 | 7,54 | 0,021 |
| | <u>Activity</u> | | | | | |
| | Activity | F(1, 12) | 1,17 | > 0.1 | 1,65 | > 0.1 |
| | Experience | F(1, 11) | 12,49 | 0,005 + | 10,919 | 0,007 + |
| Activity | F(1, 11) | 1,491 | > 0.1 | 2,133 | > 0.1 | |

Only models with interaction effects with a P-value of less than 0.10 are reported, when no such interaction effects were found on either of the types of reversals, only the main effects of the models are shown. P-values of less than 0.1 and concerning the trait anxiety factors are indicated in bold.

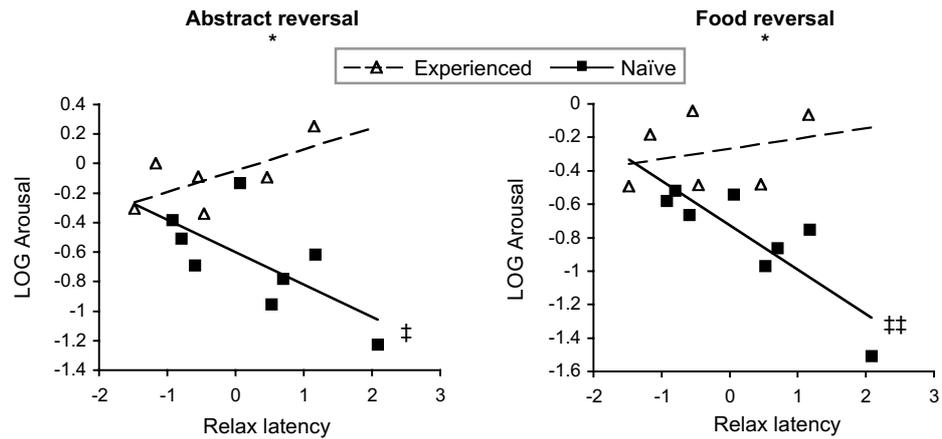


Figure 4.5. The relation between the trait anxiety factor relax latency and the LOG arousal, for the abstract reversals and food reversals (Interaction: * $p \leq 0.05$; ** $p \leq 0.01$; Separate post-hoc analysis: ‡ $p \leq 0.1$; ‡‡ $p \leq 0.01$).

DISCUSSION

The goal of this paper is to test the hypothesis that individual differences in trait anxiety negatively affect performance and behaviour in a cognitive test.

We predicted that high scores on measures of trait anxiety would be related to a low willingness to enter the learning compartment in the testing procedures. Additionally, it was expected that high scores on measures of trait anxiety would be related to reduced performance and attention for the test, and to decreased levels of arousal-related behaviour.

In summary we found that: (1) high trait anxiety subjects were reluctant to participate in the DR-test; (2) high trait anxiety subjects showed reduced attention for the DR-test; (3) high trait anxiety was associated with reduced performance; (4) the interaction of trait anxiety and previous test experience was related to the behavioural arousal of animals during the test.

First, the relations between trait anxiety, the willingness to enter the learning compartment, and the performance in the DR-test will be discussed. Next the relations between individual trait anxiety and behaviour in the DR-test will be dealt with. Finally, we will raise more general points with regard to the effects on performance and behaviour in the DR-test, and address some methodological limitations of this study.

Effect of trait anxiety on test order of the DR-test

Trait anxiety, measured as an increase in the factor activity in reaction to a loud noise, is related to the willingness to co-operate in the DR-test procedure (Figure 4.2). This result is in agreement with our expectations. So, individual differences in anxiety, as measured here, can indeed be considered as an indication of the subjects' trait anxiety (Eysenck, 1997). Itoh (2001) assessed individual differences of group housed monkeys in the willingness to approach a human experimenter to obtain a food reward. In that paper the results were interpreted as indicating an individual trait concerning the 'approach-avoidance conflict'. Such an approach-avoidance conflict was also induced by the test procedures of the present study, in which subjects had to approach the experimenter. Our results extend those of Itoh by showing that these individual differences are related to an expression of trait anxiety in a different context. Moreover, Ayers and colleagues (2001) reported that only a small portion of a socially housed group of long-tailed macaques engaged in a training paradigm to perform individual tests of their cognitive capacities. Our results suggest that individual differences in trait anxiety is one factor distinguishing "slackers" (Ayers et al., 2001) from performers.

Effect of trait anxiety on performance in the DR-test

The effect reported for trials to criterion was in the direction of our expectations. High trait anxiety was related to higher numbers of trials to reach the learning criterion. This finding is in agreement with the report by Schneider and colleagues (1991) of an association between high fearfulness and poor cognitive performance. Our result is also in agreement with findings reported in work with humans (Eysenck, 1985; Revelle, 1987; Mueller, 1992; Kumari & Corr, 1998; Persson et al., 2000; Li et al., 2004) and rodents (Ohl et al., 2002; Ohl et al., 2003). Furthermore, it is also in line with previous findings of relations between other aspects of temperament and cognitive performance in primates. Stavisky and colleagues (2000) demonstrated that sociability of subjects was positively related to cognitive performance. Likewise, Watson and Ward (1996) reported a positive effect of 'curiosity' on cognitive performance. The temperament aspects studied by these authors are considered to be negatively related to trait anxiety, that is highly sociable and 'curious' animals generally have low scores on trait anxiety (Higley & Suomi, 1989; Capitanio, 1999).

High trait anxiety was not only associated with reduced performance, but also with low levels of attention (see below). This makes it difficult to assess whether the relation between trait anxiety and performance was mediated by a reduction in attention, or whether this was a direct relation such as proposed by McNaughton (1997).

In summary, our result confirms the conclusion from previous studies that individual trait anxiety negatively affects cognitive performance.

In a previous paper (Chapter 2) we used the DR-test described here to test the hypothesis that a history of social subordination would lead to greater age-related cognitive decline compared to animals with a history of social dominance, via the deleterious effect of prolonged exposure to glucocorticoids on the brain. This hypothesis was not supported by the data, which showed relatively large variation in the young monkeys compared to the old monkeys (Chapter 2). We suggested that differences in individual temperament were related to those findings. The relation between trait anxiety and performance reported here supports that suggestion. Differences in trait anxiety may have increased inter-individual variation among young animals, and may have had a confounding effect on the inter-individual variation among aged monkeys (cf. Touyarot et al., 2004), especially since present dominance status and trait anxiety were not related. Therefore, individual differences in trait anxiety may have confounded the inter-individual variation in the old group, hypothesised in Chapter 2. In this reasoning, we also have to consider that trait anxiety may depend on context, that is a social (e.g. social subordination) versus a non-social (e.g. responses to a loud noise) context (Lawrence et al., 1991; Fairbanks, 2001; D'Eath & Burn, 2002). Finally, the limited sample size and more importantly the small number of old subjects, do not allow us to separate of the life-time effects of subordination and trait anxiety.

Effect of trait anxiety on behaviour in the DR-test

Attention

In line with our expectations, anxious monkeys attended to the cognitive task less than their less anxious conspecifics. At first this seems to contradict results of the only other study relating differences in individual temperament to attention for a discrimination test in monkeys (Joly et al., 2001). Joly and colleagues reported that this relation was not significant. However, they suggested that this was due to a very low level of attention displayed by all subjects. The negative relation between trait anxiety and attention reported here can not, therefore, be considered to contradict the result of Joly et al. (2001).

The very general behavioural measure used here to indicate attention for the task is difficult to compare with the detailed way in which divided, sustained or selective attention are often quantified in studies of human subjects, for instance in milliseconds of response time (e.g. Eysenck, 1992; Chajut & Algom, 2003). Yet, the present results in combination with the previous finding that low levels of attention are related to poor performance (Chapter 3) do not accommodate the suggestion of improved selective attention in high anxiety subjects (Chajut & Algom, 2003). Rather, they are in line with theories of increased distractibility and of limited resources (Eysenck, 1992), which predict a deleterious effect of anxiety on the level of attention.

Arousal

We expected to find a negative relation between high trait anxiety and the level of behavioural arousal during the DR-test. In line with our expectations, naïve subjects showed a decrease of arousal in the DR-test with a rise in the factor relax latency. Experienced subjects, however, showed no significant effect (Figure 4.5).

These results are in agreement with the discussion concerning the emotional basis of the arousal-related behaviours presented in Chapter 3. There we suggested that the basis for these behaviours in the context of the DR-test could lie in excitement related to the task rather than in anxiety related to the test procedures. According to this reasoning, high levels of trait anxiety would decrease the excitement for the task. Experienced subjects showed relatively high levels of excitement, irrespective of their trait anxiety. However, for naïve animals high trait anxiety was related to low excitement.

The explanation for this difference between the experienced and naïve animals comprises two related aspects. First of all, the experienced animals were probably more familiar with all aspects of the test procedure than the naïve subjects, despite the extensive training period. Due to this greater familiarity not even the experienced subjects with high trait anxiety were anxious about the procedures. Thus, anxiety about the procedures did not subdue their excitement for the test (i.e. arousal). The other aspect posits that especially the familiarity with a similar cognitive test differentiated the experienced from the naïve subjects. Probably, this greater familiarity with the test elevated the excitement for the test for the experienced animals.

Interpretation and conceptual remarks

Few significant results were found for both abstract and food reversals. However, the non-significant effect for the other type of reversals was always in the same direction as the significant effect. Nevertheless, it should be mentioned that by interpreting these results as significant we run the risk of over-interpretation, trying to explain relations that should be considered as type I errors. Clearly, this calls for a modest interpretation of the results.

Furthermore, no consistent results were revealed for the two trait anxiety factors. Statistically these factors are unrelated to each other. So, subjects with high values on the factor relax latency are not necessarily the same as the ones with high values on the factor activity. Therefore, it is not surprising that significant effects found for one factor are not significant for the other. The fact that these factors are statistically unrelated does not necessarily mean that they have a different temperamental or behavioural basis.

Trait anxiety cannot be seen as expressed by any single factor (Aguilar et al., 2002). Furthermore, the factors obtained here to indicate differences in trait anxiety can also be interpreted within the construct of 'reactivity', which is also sometimes called 'fearfulness' (Higley & Suomi, 1989). Higley and Suomi state

that "the trait reactivity can be represented as a number of partially overlapping characteristics" (p. 154). The factors identified here are some of these characteristics (Higley & Suomi, 1989; Crepeau & Newman, 1991; Bowers et al., 1998; Capitano, 1999). Thus, although statistically unrelated, the factors used here can be interpreted as expressions of the same underlying temperament trait.

Methodological remarks

One possible explanation for the lack of significant results in this study lies in the chosen method to determine individual reactivity. The sudden, loud noise may not have provoked the proper reaction to judge individual trait anxiety. The provoked response may be a startle reflex (cf. 'acoustic startle reflex', Winslow et al., 2002), rather than an indication of trait anxiety. However, a startle reflex can also be seen as a behavioural indication of trait anxiety (Aguilar et al., 2002; Winslow et al., 2002). Obviously, a startle reflex was part of the reaction of all animals in the present experiment. These behaviours were not included in the analysis, because the videotapes did not allow reliable records of startle reflexes of the subjects.

Another possible reason is the loudness of the noise. As Bates (1989) points out, the reaction of subjects to a stimulus (i.e. noise) is dependent on the intensity of the stimulus. At very high or low intensities both low and highly anxious subjects may respond in a similar manner. Yet, there is no reason to assume that this is the case here, considering the range of responses of the subjects in the original six variables measured (data not shown).

The present sample size is not large enough to examine whether the effects of trait anxiety on performance measures are mediated by the parameters describing the behaviour of subjects in the DR-test. For instance, it is impossible to determine whether the trait anxiety affects attention which in turn influences the performance, or whether trait anxiety is directly associated with performance. The fact that such effects were found with different trait anxiety factors further complicates this analysis.

CONCLUSION

This study has confirmed the relation between trait anxiety and cognitive performance previously reported in rodents, humans and infant primates. Methodological and conceptual difficulties hamper the interpretation of the results. Yet, this study shows that individual differences in trait anxiety can be experimentally assessed in large social groups of monkeys. Furthermore, high levels of trait anxiety were associated with a reduced willingness to co-operate in experimental procedures, reduced attention for the cognitive test, and low performance. Moreover, anxiety in relation to test procedures may be attenuated in experimentally experienced subjects, as indicated by the absence of an association between high trait anxiety and low task-related excitement.

Studies of primate cognitive capacity typically deal with small sample sizes and substantial inter-individual variation. The present work stresses the importance of individual differences in trait anxiety in the assessment of cognitive capacities. Furthermore, the interaction between previous experimental experience and trait anxiety is a clear warning to use both experienced and naïve animals in such studies.

ACKNOWLEDGEMENTS

We thank Léonie Buren, Saskia Dekker, and Myrke Nieweg for assisting in the data collection and analysis. Henk Westland and Annet Louwerse provided practical assistance. Han de Vries gave valuable statistical advice.

APPENDIX

This appendix contains additional information about:

- Measures to quantify the reactions of subjects to the noise
- Results

Measures to quantify the reactions of subjects to the noise

The following six behavioural measures were derived for the observations of the behaviour of the subjects from fifteen prior to fifteen minutes after the onset of the noise.

1 Run away distance

We observed where the subjects were at the start of the noise and where their movement stopped for the first time after the onset of the noise.

An estimate of the distance the subject moved was calculated, based on five imaginary compartments in the home cage (see Figure 4.1). These compartments were further subdivided to allow a precise determination of the location of the animal. Compartments one to three were divided into four subcompartments (ground, wall-rack, scaffolding and wire mesh ceiling), the last two compartments were subdivided into ground and ceiling only. This distance was averaged for the twelve presentations of the noise. Similarly, a control value of average run away distance was calculated for starting at a moment five minutes prior to the noise. This control value was subtracted from the distance after the onset of the noise. Finally a logarithmic transformation was applied to obtain a normally distributed measure of the average distance moved by each subject in response to the noise.

2 Latency to leave flee locations

Regularly, subjects' first halt of movement after the onset of the noise was in a location normally only used in locomotion. Such locations comprised, for instance, the ceiling of the enclosure and the climbing racks attached to the walls. For the measure 'latency to

leave the flee location' we observed the time taken to leave such a location, averaged over the number of occurrences. A logarithmic transformation was applied to obtain a normally distributed measure.

This measure was not corrected for a control value because the subjects never moved to such a location after the arbitrary moments described above.

3 Behavioural changes

We calculated the rate per minute of all changes of state behaviours (Martin & Bateson, 1993) over the fifteen minutes observation time before and after the onset of the noise. We controlled for a baseline rate of behavioural changes by subtracting the rate of the control observations from the rate of the period after the noise. The result was averaged over the twelve presentations of the noise, providing an average difference in the rate of behavioural changes before and after the onset of the noise.

4 Scratching

We calculated the average rate of all occurrences of scratching per minute of time in which the subjects were not moving, after the noise onset. This rate of scratching was corrected for the value in the control period as described for the behavioural changes.

5 Activity

The duration of state behaviours indicating activity (such as: walking, running) was calculated relative to the fifteen minutes observation time after noise onset. Again the values from the control observations were subtracted, resulting in the average difference in relative duration of activity before and after noise onset.

6 Latency to self-grooming

After noise onset, we measured the latency to the first self-grooming bout. As before we corrected these values for the latency in the control observations.

Results

The aim of the present work was to relate individual differences in trait anxiety of monkeys to behaviour and performance of these monkeys in a Discrimination-Reversal (DR) learning test.

Here a full account is presented of all relations with a p-value of less than 0.1, no distinction is made between results found for abstract or food reversals; Tables 4.4-4.7 and Figures 4.3-4.5 do provide results for both types of reversals. See the Analysis section for a description of the design of the statistical analysis.

I Examination of factors of trait anxiety

The factor analysis of the six variables measured in response to the noise, provided three factors, each unrelated to the others. Together, these factors explained 88 percent of the total variation. See Table 4.1 for the factor loadings.

The first factor is mainly determined by the latency to leave a flee location and the latency to the first bout of self-grooming. Both these variables can be seen as indicating the moment at which animals return to a more normal situation. The onset of self-grooming can be interpreted as an indication of when a stressful period has ended (Aureli & Van Schaik, 1991; Spruijt et al., 1992). Thus, the latency to the first self-grooming bout is taken as an estimate of the anxiety experienced after the noise. Likewise, the immobility indicated by the latency to leave a flee location has also been related to anxiety (Higley & Suomi, 1989). We will, therefore, interpret this factor as indicating the latency to recover after the noise, or in other words a *relax latency*.

The main factor loadings of second trait anxiety factor come from the rate of behavioural changes and the relative duration of activity. Thus, this factor can be seen as signifying the level of activity of the subject in the fifteen minutes after noise onset (Crepeau & Newman, 1991; Capitanio, 1999; Kaplan et al., 2001; Aguilar et al., 2002). We shall call this factor *activity*.

The third trait anxiety factor, labelled 'distance', is primarily determined by the distance moved by the subjects after the onset of the noise and, inversely, by the scratching rate. This relation of a greater distance and a reduced scratching rate over fifteen minutes of observation makes this factor more difficult to interpret in the sense of trait anxiety. In view of this interpretation difficulty, and because the goal of this paper is to test hypotheses concerning the relation between trait anxiety and cognitive performance, we will focus on the effects of the first two trait anxiety factors.

So, two factors were further used to investigate the relation between individual trait anxiety and performance and behaviour in the DR-test. These factors were not associated with either present dominance status or age of the subjects (Table 4.2).

II Effect of trait anxiety on test order of the DR-test

The results of these analyses are given in Table 4.3.

First, we tested whether the test order depended on experience, present dominance status or age. Experienced subjects preceded naïve subjects in the test order. When age was excluded, an interaction between experience and present dominance status approaching significance (Table 4.3, Figure 4.2) was found. When this interaction effect was also excluded, and the model was tested with only the main effects of experience and present dominance status, only the effect of experience remained significant (Table 4.3).

We expected that subjects with high levels of trait anxiety would be later in the test order. We first tested whether the trait anxiety factors alone affected the test order. No effects were revealed for either trait anxiety factor.

Then, we constructed a model to test whether the trait anxiety factors had an effect in addition to the effects of experience and present dominance status. For the factor relax

latency, two interaction effects were found. Experienced subjects entered the learning compartment later if they had a low score on the factor relax latency, while naïve subjects showed the opposite effect. Additionally, the effect of present dominance status was negative for experienced subjects and positive for naïve animals (Table 4.3).

The latter interaction was also found in the analysis of the factor activity. Furthermore, the effect of the factor activity proved to be significant and positive (Table 4.3, Figure 4.2) in addition to this interaction effect. Thus, subjects with a high level of the factor activity were less willing to enter the learning compartment than other subjects did.

III Effect of trait anxiety on performance in the DR-test

Effect of trait anxiety on the number of trials

High levels of trait anxiety were expected to be related to a lower cognitive performance, i.e. to high numbers of trials to criterion. The results of these analyses are given in Table 4.4.

First we tested whether the trait anxiety factors alone were associated with the number of trials to reach the learning criterion. No effects were revealed except for a positive effect of the factor activity, which approached significance. Subjects with a high score on the factor activity required more trials to reach the learning criterion than other subjects did.

In the following models, we added the previous test experience and age of the subjects because these variables had previously been shown to affect the trials to criterion (Chapter 2). We examined whether the trait anxiety factors had an additional positive effect on the number of trials over and above the effect of these variables.

First we tested whether any of the three interactions yielded significant results. An interaction approaching significance was found between experience and the factor relax latency (Table 4.4). Experienced subjects needed fewer trials when they had a long relax latency than when they had a short relax latency. The naïve animals showed the inverse effect.

Further, post-hoc analyses revealed that, when tested separately, the effect of the factor relax latency on the trials to criterion was not significant for either experienced ($F(1, 4) = 3.34, p > 0.1$) or naïve ($F(1, 6) = 3.13, p > 0.1$) animals. The model without this interaction provided no significant result (Table 4.4).

For the factor activity high scores were still associated with a high number of trials, even in addition to the effects of experience and age (Table 4.4, Figure 4.3).

Effect of trait anxiety on the percentage of perseverative errors

High levels of trait anxiety were expected to be related to a lower cognitive performance, i.e. to a high percentage of perseverative errors. The results of these analyses are given in Table 4.5.

First, the trait anxiety factors alone were not associated with the percentage of perseverative errors. In the following models, we added the present dominance status of the subjects, because this variable had previously been shown to affect the percentage of

perseverative errors (Chapter 2). We examined whether the trait anxiety factors had an additional positive effect. In contrast to our expectations, no effects of the factors relax latency or activity were revealed.

IV Effect of trait anxiety on behaviour in the DR-test

Effect of trait anxiety on attention

High levels of trait anxiety were expected to be related to a reduced level of attention for the DR-test. The results of these analyses are given in Table 4.6.

First we tested whether the trait anxiety factors alone were associated with the level of attention for the DR-test. Neither of the trait anxiety factors had an effect.

In the following models, we added the present dominance status of the subjects because this variable had previously been shown to affect the level of attention (Chapter 3). The interaction effects did not yield significant results, thus we considered the effects of the main factors. The factor relax latency had an additional significant negative effect on the level of attention (Table 4.6, Figure 4.4). No effect of the factor activity was revealed.

Effect of trait anxiety on arousal

High levels of trait anxiety were expected to be related to reduced behavioural arousal during the DR-test. The results of these analyses are given in Table 4.7.

First we tested whether the trait anxiety factors alone were associated with arousal. Subjects with a high score on the factor relax latency showed less behavioural arousal in the DR-test. The factor activity did not influence the behavioural arousal.

In the following models, we added the previous test experience of the subjects because this variable had previously been shown to affect the level of arousal (Chapter 3). We examined whether the trait anxiety factors had an additional negative effect.

A higher level of arousal was found for experienced subjects with a longer relax latency, while the naïve subjects showed the reverse relation (Table 4.7, Figure 4.5). Because this interaction effect is significant we cannot interpret the main effect of either experience or relax latency without referring to the other. Further, post hoc analysis revealed that the effect for the experienced animals was not significant (Abstract: $F(1, 4) = 3.05, p > 0.1$; Food: $F(1, 4) = 0.33, p > 0.1$). However, the negative effect for the naïve animals was significant (Abstract: $F(1, 6) = 5.16, p = 0.064$; Food: $F(1, 6) = 15.91, p = 0.007$). Naïve subjects with high scores on the factor relax latency showed less behavioural arousal than naïve subjects with low scores on this factor did. The factor activity was not related to the behavioural arousal.

CHAPTER 5

Differences in reversal learning in monkeys in relation to stimulus attributes

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ABSTRACT

In investigations on cognitive abilities of primates pictures are often used. These can be abstract figures or pictures of objects. We found that the nature of pictures may greatly influence results. Contrary to our expectation subjects required more trials to reach the learning criterion with realistic pictures of food items than with abstract visual stimuli. Food stimuli were also associated with a high percentage of perseverative errors on following reversals. These relatively high numbers of perseverative errors were accompanied by high rates of arousal, possibly an effect of frustration.

We suggest that physical aspects of the stimuli, such as colourfulness and shape, overruled the possible effect of the meaningfulness of stimuli.

INTRODUCTION

In studies investigating the learning and memory capacities of primates two-dimensional stimuli are commonly used (Bartus et al., 1979; Fobes & King, 1982; Moss et al., 1988; Overman et al., 1990; Dias et al., 1996a; Voytko, 1999). In some studies 'abstract' figures or patterns were used (e.g. Moss et al., 1988), whereas in others pictures of objects were used (e.g. Overman et al., 1990; Voytko, 1999). Non-human primates have the ability to recognise objects which are represented in two-dimensional pictures (for a review see Bovet & Vauclair, 2000; Fagot et al., 2000). In addition, it has been demonstrated that social stimuli (i.e. showing other conspecifics) are preferred as rewards in cognitive tests (for a review see Anderson, 1998).

Taken together, these results raise the question whether the nature of stimuli used influences the results of cognitive tests with primates. Do stimuli depicting biologically relevant objects yield better results? For instance, subjects may learn a discrimination faster when they can recognise the relevant objects depicted in stimuli.

Some previous work has been done comparing social and abstract stimuli in this respect but no firm conclusions can be drawn from that (Rosenfeld & Van Hoesen, 1979; Dittrich, 1994; Anderson, 1998). In this paper we report a study comparing performance of reversal learning in a Discrimination-Reversal test with monkeys using either abstract or biologically relevant stimuli.

Leavens and colleagues (2001) summarised the relation between behavioural and physiological measures of arousal (Schino et al., 1996) on the one hand, and on the other hand, physiological arousal and task difficulty (Gotthardt et al., 1995). This led to the expectation that increased task difficulty is associated with elevated levels of arousal. In concert with their expectation, Leavens et al. (2001) reported that chimpanzees (*Pan troglodytes*) faced with an increase in test difficulty showed behavioural evidence of emotional arousal. We also examine the behaviour of our subjects shown during this test, and predict that high behavioural arousal is associated with a difficult task.

This study was part of a larger project investigating age-related cognitive decline in relation to the life time dominance history of subjects. Therefore, the present analyses included between-subject factors derived from that project (Chapter 2).

In sum, we expect improved performance and lower rates of emotional arousal for the biologically relevant stimuli. We do not expect to find an interaction between this difference in performance and between-subjects variables.

METHODS

Subjects

Two groups of socially housed long-tailed macaques (*Macaca fascicularis*) participated in this study, the R- and the L-group. Both groups were housed at the Ethology Station of Utrecht University; for more information on the groups and their housing see Chapter 2. Fifteen female subjects were selected, nine from the R-group and six from the L-group. Six of the selected females from the R-group had previously participated in a pilot study four years earlier (Veenema, 1998).

Experimental set-up and testing procedure

Testing was conducted in an experimental wire mesh cage, situated in a room adjacent to the animals' home cage. The experimental cage contained a learning compartment in which subjects could be separated from the group (auditory, olfactory and limited visual contact remained possible). The learning compartment could be used as a lock system between the experimental cage and the, preferred, home cage. For more information on this set-up and the testing procedure see Chapter 2 and Figure 2.1.

In the learning compartment subjects were presented with the test, using a touch screen. The test and the delivery of food rewards were operated by a computer. Rewards were delivered into a small cup centred underneath the touch screen.

The experimenter left the room after starting the test for a subject. A video camera mounted above the learning compartment allowed the experimenter to monitor and observe the behaviour of the subjects in an adjacent room. Behavioural elements were directly logged into a computer file using the Observer® programme.

Both groups received extensive training in the procedures of separation and testing before testing began, to ensure that the animals would enter the learning compartment voluntarily. In the final part of the training all subjects were given a pre-test discrimination task in which they were familiarised with the discrimination test.

Discrimination-Reversal test

In the learning compartment subjects performed on a Discrimination-Reversal (DR) test. Each trial consisted of the same four pictures (see next section) being displayed on the four sides of the touch screen. The rest of the screen was black throughout the test. The configuration of the pictures was established in a pseudo-random manner such that each picture was displayed equally often in each location within each session of 50 trials.

Subjects had to learn which stimulus was consistently associated with a reward (for more details on this test, see Chapter 2). When this initial discrimina-

tion had been learned, up to a pre-set learning criterion (nine correct out of ten consecutive trials), the association with the reward was switched (reversed) to one of the other stimuli. Such a reversal was done each time a subject reached the learning criterion. Subjects received a maximum of 50 trials per day and could use more than one day to reach the pre-set learning criterion.

Pictures

Two types of stimuli were used. Two of the four stimuli, which we will call 'food' stimuli, depicted food items well known to and preferred by the subjects, namely an apple and a piece of carrot. Digital photographs were used to create these food stimuli. The other two stimuli consisted of meaningless, abstract shapes of various colours, which we will call 'abstract' stimuli (Figure 5.1). The food stimuli were placed on a grey background measuring five by five centimetres; the abstract stimuli were placed on a black background (i.e. the same as the rest of the screen) of the same dimensions. The order in which the four stimuli were designated as the reward stimulus was fixed and the same for all individuals, namely: first one of the abstract pictures, second the carrot picture, third the other abstract picture and last the apple. This way each reversal with an abstract picture as the correct stimulus was followed by a reversal with a food pictures as the correct stimulus, and vice versa.

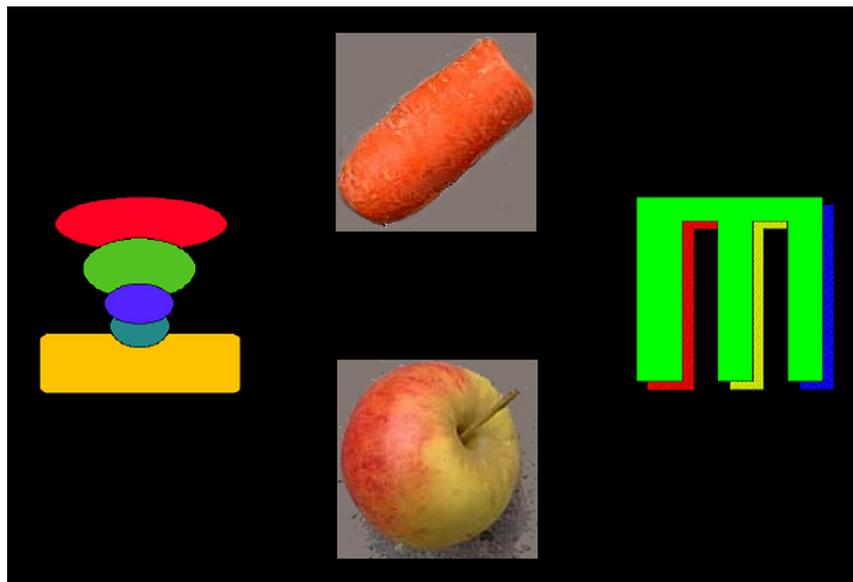


Figure 5.1. Example of stimuli presentation on the Discrimination-Reversal test (see text for details)

We will refer to a reversal on which a food stimulus becomes the correct stimulus as a 'food reversal', likewise 'abstract reversal' means that an abstract picture will be correct (and thus that choosing one of the food stimuli constitutes a perseverative choice, see below).

Measures

All subjects, but one, attained criterion on the initial discrimination task and four consecutive reversals. The subject who did not reach criterion on four reversals (Yukka) consistently responded to one side of the screen for over 2000 trials. Therefore, data of this female were ignored in all analyses.

Performance measures

The performance of the subjects was measured in different ways. First, the number of trials a subject needed (trials without a response were omitted) to reach the learning criterion on a certain task is called the *trials to criterion* on that particular task. In our analysis we used a logarithmic transformation of this measure, because the data were not normally distributed.

Secondly, the pattern of errors made was analysed. On every trial subjects can choose between four stimuli. Three of these constitute wrong choices. One of these is a perseverative error: a response to the previously rewarded stimulus. A choice for other picture of the same type as the perseverative error is the second possible wrong choice. The last possibility is for subjects to choose the incorrect stimulus of the same type as the correct stimulus.

The number of perseverative mistakes a subject made before she reached the learning criterion on a certain task is expressed as a percentage of the total number of trials to criterion she needed on that task. This is called the *percentage of perseverative errors* on that task.

Similarly we calculated the number of choices made for the incorrect stimulus of the same type as the perseverative error stimulus. This was likewise expressed as a percentage of the trials to criterion; we labelled this the *percentage other of perseverative type*.

Finally, the number of choices made for the incorrect stimulus of the same type as the correct stimulus was calculated, and again expressed as a percentage of the trials to criterion. We called this the *percentage other of correct type*.

Behavioural measure

Leavens and colleagues (2001) reported higher occurrences of displacement behaviours with increasing task difficulty. For our measure of behavioural arousal we calculated the summed rate per minute of testing time of the occurrence of the following displacement behaviours: scratching, yawning, body shaking, barbiting and self-grooming. We used a logarithmic transformation, because the data were not normally distributed. As we have argued previously (see Chapter 3) this measure of behavioural arousal should be interpreted with

caution regarding the underlying emotional basis. Therefore, we regard these displacements behaviours as a neutral indication of emotional arousal during testing.

Analyses

The goal of this paper is to compare performance and behaviour associated with different types of stimuli in a Discrimination-Reversal (DR) test. As we used a DR-test with a single, initial discrimination we can only analyse differences between types of reversals (i.e. abstract versus food reversals).

Fourteen of the animals reached the pre-set learning criterion on four reversals, thus there were four data points for each subject. Obviously, the data points of one subject cannot be considered to be independent of one another; therefore, we tested within-subject effects with a repeated measures design in all statistical tests.

First, we tested the within-subjects effect of stimulus type (abstract or food) and task number (one or two) for all dependent variables.

Next, we examined the interaction of these effects with between-subject factors. This study was part of a larger project investigating age-related cognitive decline in relation to the dominance history of subjects. The between-subject factors tested here were derived from this project (for more information see Chapter 2). The factor 'experience' indicated whether subjects have had previous experimental experience with a comparable cognitive test. 'Dominance history' categorises the subjects into three classes describing the life-time history of their dominance status as high, middle or low ranking. Finally, we tested the 'age' of the subjects as a between-subjects co-variable. To test the effect of these variables we followed the same strategy as in Chapter 2. We first tested whether the interaction between dominance history and age affected the model of the within-subjects effects. If this interaction effect was not significant it was dropped from the model and only the main between-subjects effects were tested.

RESULTS

This paper aims to compare two types of stimuli in terms of performance on and arousal during a Discrimination-Reversal (DR) test.

Within-subject effects of stimulus type and task number

In contrast to our expectations subjects needed more trials to reach criterion on the reversals with the biologically relevant, food stimuli than with the abstract stimuli. Furthermore, this effect became larger for task number two (Table 5.1, Model I and Figure 5.2A). Thus, reversals with the food stimuli as correct stimulus were more difficult than the abstract stimuli.

The opposite relation was found for the perseverative errors. Subjects persevered more on the reversals with the abstract stimuli as correct, than on the

Table 5.1. F- and P- values of General Linear Linear Models for repeated measures testing within- and between-subject effects

| Independent variables | D.F. | Dependent variables | | | | | | | | | | |
|---------------------------------|---------|---------------------|--------------|--------------|--------------|------------------|--------------|------------------|--------------|---------|--------------|--|
| | | No. of trials | | % Pers. err. | | % Other pers. t. | | % Other corr. t. | | Arousal | | |
| | | F | P | F | P | F | P | F | P | F | P | |
| <i>Within-subject effects</i> | | | | | | | | | | | | |
| Model I | | | | | | | | | | | | |
| Stim. type | (1, 13) | 41.92 | 0.000 | 41.49 | 0.000 | 30.48 | 0.000 | 154.62 | 0.000 | 4.94 | 0.045 | |
| Task no. | (1, 13) | 6.02 | 0.029 | 2.83 | > 0.1 | 0.29 | > 0.1 | 9.19 | 0.010 | 3.15 | 0.099 | |
| Stim. type*Task no. | (1, 13) | 7.03 | 0.020 | 0.02 | > 0.1 | 7.08 | 0.020 | 11.31 | 0.005 | 1.25 | > 0.1 | |
| <i>Within-subject effects</i> | | | | | | | | | | | | |
| Model II | | | | | | | | | | | | |
| Stim. type | (1, 9) | 47.10 | 0.000 | 48.73 | 0.000 | 30.61 | 0.000 | 532.11 | 0.000 | | | |
| Task no. | (1, 9) | 3.62 | 0.089 | 2.05 | > 0.1 | 0.35 | > 0.1 | 14.06 | 0.005 | | | |
| Stim. type*Task no. | (1, 9) | 9.94 | 0.012 | 0.03 | > 0.1 | 4.81 | 0.056 | 8.51 | 0.017 | | | |
| Stim. type*Exp. | (1, 9) | 0.93 | > 0.1 | 0.08 | > 0.1 | 0.58 | > 0.1 | 0.01 | > 0.1 | | | |
| Task no.*Exp. | (1, 9) | 1.38 | > 0.1 | 1.39 | > 0.1 | 1.08 | > 0.1 | 0.93 | > 0.1 | | | |
| Stim. type*Task no.*Exp. | (1, 9) | 4.52 | 0.062 | 0.01 | > 0.1 | 0.48 | > 0.1 | 1.36 | > 0.1 | | | |
| Stim. type*Dom. | (2, 9) | 1.81 | > 0.1 | 2.45 | > 0.1 | 0.75 | > 0.1 | 16.06 | 0.001 | | | |
| Task no.*Dom. | (2, 9) | 2.49 | > 0.1 | 0.27 | > 0.1 | 0.06 | > 0.1 | 5.33 | 0.030 | | | |
| Stim. type*Task no.*Dom. | (2, 9) | 4.25 | 0.050 | 0.12 | > 0.1 | 0.31 | > 0.1 | 0.81 | > 0.1 | | | |
| Stim. type*Age | (1, 9) | 0.20 | > 0.1 | 0.44 | > 0.1 | 2.20 | > 0.1 | 3.70 | 0.087 | | | |
| Task no.*Age | (1, 9) | 0.05 | > 0.1 | 0.20 | > 0.1 | 0.16 | > 0.1 | 0.64 | > 0.1 | | | |
| Stim. type*Task no.*Age | (1, 9) | 0.29 | > 0.1 | 0.49 | > 0.1 | 0.36 | > 0.1 | 0.26 | > 0.1 | | | |
| <i>Between-subjects effects</i> | | | | | | | | | | | | |
| Exp. | (1, 9) | 20.16 | 0.002 | 21.01 | 0.001 | 0.43 | > 0.1 | 36.47 | 0.000 | | | |
| Dom. | (2, 9) | 1.01 | > 0.1 | 0.86 | > 0.1 | 0.21 | > 0.1 | 6.13 | 0.021 | | | |
| Age | (1, 9) | 5.19 | 0.049 | 0.28 | > 0.1 | 0.47 | > 0.1 | 0.01 | > 0.1 | | | |

Stim. - stimulus; no. - number; exp. - experience; dom. - dominance history; pers. - perseverative; err. - error; t. - type; corr. - correct
P-values of less than 0.1 are indicated in bold.

food reversals (Table 5.1, Model I and Figure 5.2B). So after taking longer to learn the discrimination (i.e. associate the stimulus with the reward) on the food tasks, subjects also needed longer to disassociate this stimulus with the reward. The analysis of the other two types of error revealed that on food reversals subjects made significantly more choices for the other item of the correct type than on the abstract reversals (percentage other of correct type, Table 5.1, Model I and Figure 5.2C). Moreover, on abstract reversals subjects chose the other item of the perseverative type significantly more often than on food reversals (percentage other of perseverative type, Table 5.1, Model I and Figure 5.2D).

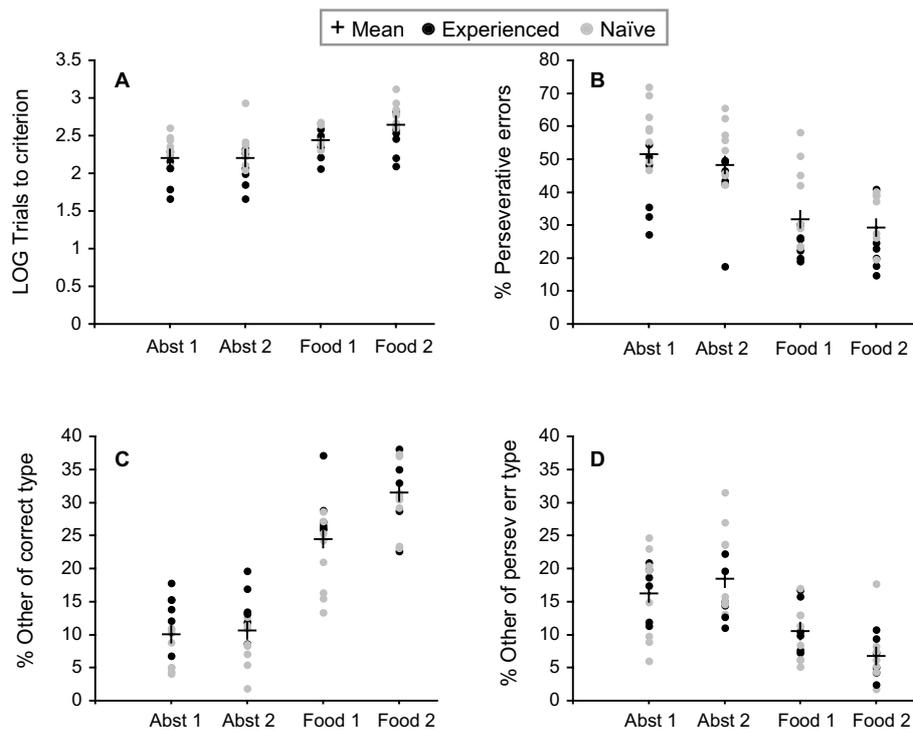


Figure 5.2. Stimulus type and task number in relation to the log trials to criterion (A), percentage of perseverative errors (B), percentage of other of correct type (C), and percentage of other of perseverative type (D)

Thus, it appears that subjects find the food stimuli more difficult to discriminate than the abstract stimuli. Once, they have mastered this discrimination and the stimulus-reward association is reversed to an abstract stimulus, subjects persevere longer with the type of stimulus that was previously correct. When after a reversal, subjects no longer receive rewards for the previously correct food

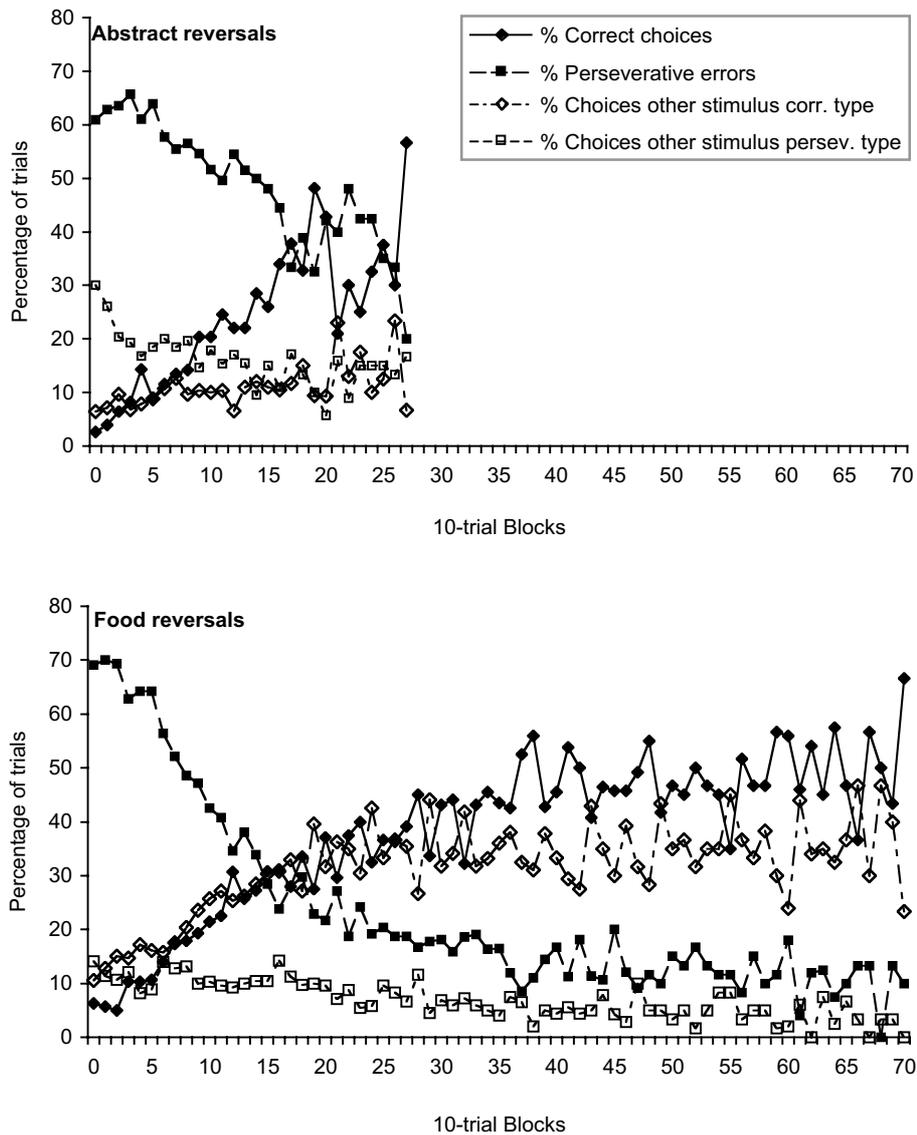


Figure 5.3. Progression of the performance on abstract reversals and food reversals. The performance of subjects on reversals was divided into blocks of ten trials (X-axis). For each 10-trial block the percentage of correct choices, the percentage of perseverative errors, percentage of choices for the other stimulus of the correct type, and the percentage of choices for the other stimulus of the perseverative type were calculated. The total number of 10-trial blocks subjects needed to reach criterion varied with their performance, thus the number of subjects represented in the datapoints decreases from left to right; datapoints represent averages for at least three subjects.

stimulus, they may be confused and try the other food stimulus before switching to the abstract stimuli (see Figure 5.3).

Effects of between-subjects factors

The results of the analysis of the between-subjects effects were in line with those reported earlier (Chapter 2). The life time dominance history did not have an additional negative influence on the effect of age for any of the within-subject effects studied here. However, a near significant interaction effect for an interaction between stimulus type, task number, dominance history and age was found for the trials to criterion ($F(2, 7) = 3.58, p = 0.085$). The regression lines for the effect of age on the performance were not parallel for the three dominance classes. Furthermore, the slopes of these lines differed for the stimulus types and task number (data not shown). However, in no instance did these lines diverge with increasing age to show an effect of dominance history on age-related cognitive decline (see also Chapter 2).

The previous experience of subjects was associated with superior performance (Table 5.1, Model II), while old age was related to a high number of trials to reach the pre-set learning criterion (Table 5.1, Model II).

The difference in performance between the two stimulus types was not dependent on the age of the subject, although this interaction approached significance for the percentage of choices for the other stimulus of the correct type (Table 5.1, Model II).

Several effects of the dominance history of subjects were found. A history of low dominance rank was associated with a high percentage of choices for the other stimulus of the correct type (Table 5.1, Model II). Additionally, significant interactions between dominance history and this variable as well as between dominance history and trials to criterion were found. These results suggest that differences in performance between the stimuli depended on the dominance status of the subjects (Table 5.1, Model II).

In line with the analysis of our previous report (Chapter 2) we re-analysed the effects of the between-subjects factors, using the present dominance status of subjects at the start of the test, instead of their life time dominance history. None of the effects of dominance history mentioned above remained significant. Only a tendency for an interaction between present dominance status and task number was found ($F(2, 9) = 3.50, p = 0.075$; data not shown).

Arousal

In contrast to our expectation subjects showed higher rates of arousal-related behaviour during the more easy reversals, that is during reversals which require fewer trials to criterion, namely the abstract reversals (Table 5.1, Model I and Figure 5.4). No effect of task number was found.

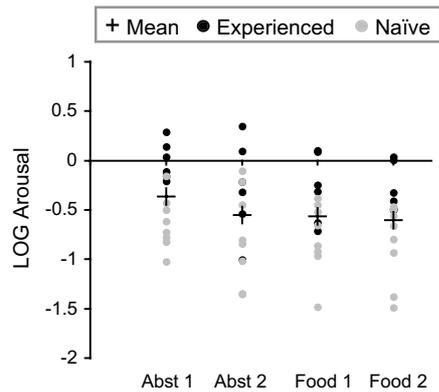


Figure 5.4. Stimulus type and task number in relation to arousal

DISCUSSION

Within-subject effects of stimulus type

In this study, long-tailed macaque females performed on a four-choice Discrimination-Reversal test in which they had to form an association between a reward and either a food or an abstract stimulus. Subjects needed more trials to reach the pre-set learning criterion when a picture depicting a preferred food-item was the rewarded stimulus than when an abstract, meaningless picture was the rewarded stimulus. Assuming that the subjects did recognise the depicted objects in the food stimuli this result is counter-intuitive. Three possible explanations for this result will be considered.

One possible explanation for this is that the animals did, in fact, not recognise the food items depicted in the stimuli. The present study was based on literature confirming the notion that primates can recognise objects depicted in two dimensional stimuli (for an extensive review see Bovet & Vauclair, 2000; Fagot et al., 2000). However, the present data do not allow conclusions to be drawn in this respect.

If we assume the food items were recognised, generalisation of these stimuli may be another explanation for the results. Subjects may have classified both food stimuli as 'food' rather than distinguishing between the pictures. As a result subjects may easily make the distinction between 'food' and the abstract stimuli, while discriminating between the two food items is more difficult, as both represent food.

The present data do not allow us to separate this possibility from a third possible explanation. This last possibility is independent of whether the objects represented in the stimuli were recognised or not. Cole (1953; and others, see Fobes & King, 1982) and Tomonaga and Matsuzawa (1992) showed that colour

and outer contourlines are important determinators of success in discrimination tests. Obviously, the food stimuli used here were more similar in colour and contrast than the abstract stimuli. Also, both food stimuli were placed on a grey background of equal size and shape, whereas the abstract stimuli had different outer shape (these were placed on a black background). These differences in shape and colour of the two kinds of stimuli may have affected the ease with which they can be distinguished. Thus, the food stimuli may be less physically contrasting and, therefore, more difficult to distinguish than the abstract stimuli based on their physical properties (cf. Kornowski & Petersik, 2003).

These latter two possible explanations both predict that the animals have more difficulty in discriminating between the two food stimuli than between the abstract stimuli. Also, these explanations are equally supported by the results of this study. We showed that subjects frequently chose the other food stimulus when a food stimulus was correct, and that they did so more often than choosing the other abstract stimulus when an abstract stimulus was correct. In other words, subjects had more difficulty in choosing, or discriminating between the food stimuli than between the abstract stimuli. In Figure 5.3 the lines of the percentage of correct choices and the percentage of choice for the other stimulus of the correct type diverge (after seven 10-trial blocks) for the abstract stimuli while they do not for the food stimuli.

Additional research is needed to examine the circumstances under which semantic information of a stimulus does improve discrimination performance of primates.

In the present study subjects needed longer to associate the food stimuli with a reward than the abstract stimuli. Moreover, once they have formed this association they need more trials to extinguish it when the reward contingency has been reversed. This finding suggests that the longer period needed to initially establish the association between the stimulus and the reward for the food stimuli causes a stronger reinforcement value for this stimulus than when one of the abstract stimuli is rewarded. Subsequently, on the following reversal the animal needs more time to extinguish this strong reinforcement value. Thus, the subjects continue to make perseverative errors for a longer period of time. Additionally, the difficulty our subjects had with discriminating between the food stimuli may have contributed to this. This difficulty may have caused subjects to become 'confused' after such a reversal resulting not only in more perseverative errors but also in more errors made to the other food picture (also incorrect). Figure 5.3 shows that the percentage of choices for the other stimulus of the perseverative type starts relatively high for the abstract reversals, only to reach a similar level as for the food reversals after fifteen 10-trial blocks.

In summary, when subjects start with a food reversal, i.e. a food stimulus becomes the rewarded picture, they quickly learn that one of the food stimuli is now rewarded rather than one of the abstract stimuli. However, the animals need

many trials to learn which of the food stimuli is the rewarded stimulus. Once they have reached the pre-set learning criterion, the reward relation is reversed to one of the abstract stimuli. Now, subjects do not receive rewards for the food stimulus they have just learned was the correct one. Subjects keep on trying for an extended period whether one of the food stimuli is rewarded. They make many choices for the both food stimuli. Once they have learned that one of the abstract stimuli is now being rewarded, and not one of the food stimuli, they very quickly learn which of the abstract stimuli is correct.

This pattern of correct and wrong choices is likely to be based on the good physical contrast between the food and abstract stimulus types, and on the other hand, the poor distinctiveness of the two food stimuli. Nevertheless, an additional generalisation effect between the two food stimuli cannot be excluded.

Within-subject effects of task number

In addition to the effects of stimulus type, task number was also shown to affect performance, in interaction with the stimulus type. More trials were needed to reach the learning criterion on the second food reversal than on the first. Similarly, the percentage of choices for the other stimulus of the correct type was higher on the second than on the first food reversal. These results suggest that subjects had greater difficulty discriminating between the food stimuli on the second reversal than on the first. This is possibly due to the effect of proactive interference (review: Wright et al., 1986), in which earlier learned stimulus-reward associations interfere with the learning of later associations. The fact that no such difference in task number was found for the abstract reversals suggests that the influence of proactive interference may be dependent on the 'dissimilarity' of stimuli. Also, this effect may be due to the stronger reinforcement value for the food stimuli (see above).

Interactions with experience, dominance history and age

This study was part of a larger project investigating the effect of life time dominance history on age-related cognitive decline (Chapter 2). In the present investigation we also examined whether experience, age, life time dominance rank, or the interaction of these influenced the effects of stimulus type and task number. The within-subject effects were not significantly affected by these between-subjects factors. So, the increased difficulty of the food stimuli was comparable for all subjects. This is in concordance with the results of Leonards and colleagues (2002) that the age-related cognitive decline in memory performance of humans is the same for relevant stimuli (faces) than for non-relevant stimuli (doors).

Arousal

The finding that animals showed higher level of arousal, in the form of a higher rate of displacement behaviours, on tasks for which they required less trials to reach criterion appears to be in contrast with the report by Leavens and colleagues (2001). These authors reported that such behaviour of a chimpanzee (*Pan troglodytes*) was related to an increase in task difficulty. However, when considering our results in relation to the discussion above concerning the perseverative errors, this apparent contradiction disappears. When subjects start the task with one of the abstract pictures as the rewarded stimulus (the 'easier' tasks), they relatively often respond to the previously correct food stimulus, which is now incorrect, expecting to receive a reward. The frustration that accompanies this leads to the behavioural arousal shown. Also, the confusion described above may contribute to the frustration of the subjects since they now respond to the other stimulus of the type that was previously correct expecting to get a reward. Therefore, our results that less difficult cognitive tasks are related to higher rates of displacement behaviours, do not contradict the report by Leavens et al.(2001). They are in line with the reasoning of Leavens and colleagues, as well as Itakura (1993), in the sense that animals exhibit a higher rate of emotional arousal in tasks in which they experience higher levels of frustration. Similarly, Lyons and colleagues (2000a) reported that adult squirrel monkeys (*Saimiri sciureus*) show an increase in cortisol in relation to not receiving an expected reward.

Elsewhere, we reported that experienced subjects showed higher levels of arousal and improved performance compared to naïve subjects on both abstract and food reversals (Chapter 3). We proposed that this difference was based on increased task-related excitement of the experienced animals (Thayer, 1996; Spruijt et al., 2001). The within-subject effect reported here is in line with this suggestion; the expected, but not received, rewards stimulate the, anticipatory, excitement (cf. 'energetic arousal', Thayer, 1996) of the animals.

CONCLUSION

The aim of this paper was to test the hypothesis that the use of biologically relevant pictures in tests of cognitive function may improve performance. With respect to this hypothesis no general conclusions can be drawn from the present results. The realistic stimuli used here, showing preferred food items, were found to reduce performance. The analysis of errors suggests that the physical characteristics of these stimuli made them more difficult to distinguish from one another than the abstract stimuli used here. Also, tasks in which relatively high numbers of perseverative mistakes were made were associated with high levels of arousal, indicating frustration of not getting expected rewards.

ACKNOWLEDGEMENTS

We thank Michelle Doumen, Patricia Verschoor and Leonie de Visser for assisting in the data collection. Henk Westland and Annet Louwerse provided practical assistance. Han de Vries and Cas Kruitwagen gave expert statistical advice.

CHAPTER 6

Meaning and brightness of stimuli do not
influence discrimination learning in monkeys

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ABSTRACT

Several studies have addressed the learning capacities of animals using two-dimensional visual stimuli. This method is likely to become predominant in the future considering the possibilities of automating test procedures. However, little is known of how features of stimuli, such as colouration, affect discrimination performance. This study was aimed at testing whether meaningful or brightly coloured stimuli were more easily discriminated by monkeys than non-meaningful or dull ones. Eight female macaques were tested on a series of 21 discrimination tasks. The stimuli of these tasks varied along three variables: familiarity, biological relevance (i.e. food vs. non-food items) and colourfulness. These stimulus features did not affect discrimination performance.

These results suggest that differences in stimulus features do not explain differences in discrimination performance between different studies. Results are also discussed in light of how pictures are perceived and recognised by primates.

INTRODUCTION

Over the past decades many studies have addressed the learning capacities of animals using visual stimuli. However, little is known of how various features of these stimuli affect learning performance.

The primates have played an important role in the field of animal learning and cognition. Likewise, discrimination learning tests have been an important tool in such studies. Many of these studies examining primates used object-based discrimination tests, but visual discrimination tests using two-dimensional stimuli have been used as well. This latter method is likely to become predominant in the future considering the possibilities and advantages of automating test procedures (Bhatt & Wright, 1992; Andrews, 1994; Washburn et al., 1994; Dias et al., 1996b; Crofts et al., 1999; Fagot et al., 2000). Traditionally, the stimuli used in these visual discrimination studies were 'neutral' stimuli such as patterns or geometrical shapes (Moss et al., 1988; review: Anderson, 1998). Other studies have used stimuli depicting various objects (e.g. Overman et al., 1990: car keys, screwdriver, shoe etc.; Voytko, 1999: no details available). The stimuli used in the various studies differ in a number of aspects such as colour, shape, contrast, familiarity and relevance. However, there is only scarce information concerning the possible effects of these differences on the results of discrimination tests.

Several early studies have reported that macaques use colour more readily than form in discrimination tests (Harlow, 1945; Cole, 1953; Warren, 1954; Bartus et al., 1979; Rosenfeld & Van Hoesen, 1979; see also Fobes & King, 1982). More recently, in a study on human subjects Suzuki and Takahashi (1997) have noted that recognition memory was better for colour pictures than for black and white stimuli. Another stimulus feature was studied by Iwai and colleagues (1986); they reported that the size of the discriminative cue relative to the size of the background influences the discriminability of stimuli for macaques (see also Fobes & King, 1982).

The effect of familiarity and relevance of stimuli on discrimination have received much less attention. There is evidence that primates can recognise objects shown in pictures (reviews: Bovet & Vauclair, 2000; Fagot, 2000) and that monkeys prefer certain (socially) familiar or known stimuli over unfamiliar or unknown ones (review: Anderson, 1998).

However, we know of only one paper directly comparing familiar and relevant stimuli in a discrimination test. Rosenfeld and van Hoesen (1979) compared performance of rhesus monkeys (*Macaca mulatta*) on discrimination tests with various types of stimuli. One of the pairs of stimuli differed in the orientation of bars. Others showed different abstract shapes or faces of conspecifics (relevant). Their results show that their subjects performed equally well on these discriminations. However, one could argue that their stimuli (full face view of a conspecific) were considered threatening, which confounded performance (cf.

Kyes et al., 1992). Dittrich (1994) reported that long-tailed macaques (*Macaca fascicularis*) were better at discriminating line drawings of monkeys than at discriminating the geometrical or 'non-natural' stimuli of an earlier experiment (Dittrich, 1988). Yet, these findings may have been related to differences in task difficulty between the two experiments. In contrast to the reports of Dittrich, we found in Chapter 5 that abstract stimuli were more easily discriminated than stimuli depicting food items. However, this result is also confounded, namely by the physical features of the stimuli such as colour and shape. Finally, a suggestion for the importance of relevance comes from Leonards and colleagues (2002) who demonstrated that human working memory performance was better for faces than for doors.

In summary, colour is known to be an important feature determining the salience of stimuli in discrimination tests with primates. Yet, the importance of other factors such as familiarity, relevance or colourfulness remains uncertain.

Meanwhile, studies using discrimination paradigms to examine cognitive capacities of primates have yielded variable results. Differences in the used stimuli may be related to this variation in performance. This study was aimed at testing whether three features of stimuli affect discrimination performance.

First, familiar stimuli may be more easily discriminated by monkeys than unfamiliar ones.

Secondly, it may be that not all familiar stimuli are equally easy discriminated on the basis of their relevance or meaning. It could be easier to associate a stimulus with a reward when this stimulus shows an object that the animal has positive past experiences with (for instance, because it refers to a preferred food item). In contrast, it could be more difficult to associate a stimulus with a reward when this stimulus shows an object that the animal has no or neutral past experiences with. Thus, the discriminability of stimuli showing meaningful, food items (positive association) versus non-meaningful, non-food items (neutral association) may be better (cf. Leonards et al., 2002). This difference in meaning between familiar items will, therefore, be part of this investigation.

Thirdly, colour has been reported to affect the discriminability of stimuli. In addition, the level of contrast of the stimuli may affect the discriminability of two stimuli of the same type. The use of a few bright and contrasting colours could make differences between stimuli more salient compared to pictures with a more natural palette of colours (see Figure 6.1). In Chapter 5 the good discriminability of the abstract pictures may have been due to the bright, contrasting colours. Furthermore, recent studies in humans do suggest that an increase in contrast is associated with better recognition of pictures (Wichmann et al., 2002; Kornowski & Petersik, 2003). In this study, therefore, colourfulness of stimuli was investigated as a stimulus feature that may affect discrimination performance. The natural colours of stimuli were replaced with bright, contrasting colours. This also meant that the complexity of the different shades of colours occurring in the natu-

ral (realistic) colours was removed. We expect that stimuli with brighter colours will be more easily discriminated than stimuli with natural, dull colours.

Finally, this study was restricted to testing the discriminability of two stimuli of the same kind. That is, the discriminability of, for instance, two familiar items was compared with the discriminability of two unfamiliar items. Another approach would have been to let subjects discriminate between different kinds of stimuli, for example, a familiar and an unfamiliar stimulus. This latter approach, however, would not allow an investigation of the effect of the stimulus trait in itself on the discriminability of two stimuli, which was the aim of this study.

METHODS

Subjects

We studied the performance of long-tailed macaques (*Macaca fascicularis*) living in an established social group. They were housed in a large indoor (80 m²) - outdoor (200 m²) enclosure at the Ethology Station of Utrecht University. The group consisted of a number of adult males, females and their offspring, numbering 63 individuals in total at the start of testing. Eight adult female subjects were selected to participate, based on their co-operation during a training period. Seven of the selected females had participated in a learning test three years earlier (see Chapter 2).

Experimental set-up

Testing was conducted in an experimental wire mesh cage, situated in a room adjacent to the animals' home cage. The experimental cage contained a learning compartment in which subjects could be separated from the group (auditory, olfactory and limited visual contact remained possible). The learning compartment could be used as a lock system between the experimental cage and the, preferred, home cage. All adult animals of the group had previously been subjected to this lock system (for more information on the set-up and procedure see Chapter 2 and Figure 2.1). In the learning compartment subjects were presented with the test using a touch screen. A computer operated the test and the delivery of food rewards. Rewards were delivered into a small cup centred underneath the touch screen.

Training and testing procedures

During weekdays two test sessions were conducted: a morning session (between 09:30 and 12:30 hours) and an afternoon session (between 13:30 and 17:00 hours). Sessions were separated by at least two hours. Two regular daily feedings (Ssniff Pri, 10 mm, monkey chow) were given directly after the respective test sessions. During the training and testing period fruit could only be obtained as a reward in the training or testing procedure.

Table 6.1. Categorization and description of stimuli

| Feature Familiarity | Feature Meaningfulness | Feature Colourfulness | Cat. no. | Cat. code | Three different tasks of each cat. | Description of categories and examples of stimuli |
|---------------------|--------------------------------|-----------------------|----------|----------------------------------|---|---|
| Familiar | Meaningful (Food-item) | Realistic | 1 | F-M-R | F-M-R (a) F-M-R (b) F-M-R (c) | Original digital photographs of preferred food-items. apple, banana, carrot, peanut |
| | | Non-Realistic | 2 | F-M-NR | F-M-NR (a) F-M-NR (b) F-M-NR (c) | Same as M-F-R, but with fewer and brighter colours, less detail |
| | Non-Meaningful (Non-Food-item) | Realistic | 3 | F-NM-R | F-NM-R (a) F-NM-R (b) F-NM-R (c) | Original digital photographs of details of the homepage |
| | | Non-Realistic | 4 | F-NM-NR | F-NM-NR (a) F-NM-NR (b) F-NM-NR (c) | Same as M-NF-R, but with fewer and brighter colours, less detail |
| UnFamiliar | Objects | Realistic | 5 | UF-R | UF-R (a) UF-R (b) UF-R (c) | Original digital photographs of items which subjects were unlikely to have seen before. stapler, mittens, a wooden toy |
| | | Non-Realistic | 6 | UF-NR | UF-NR (a) UF-NR (b) UF-NR (c) | Same as NM-R, but with fewer and brighter colours, less detail |
| | Abstract shapes | 7 | UF-A | UF-A (a) UF-A (b) UF-A (c) | Simple, uniformly, coloured shapes blue triangle, green circle | |

Cat. - category; no. - number

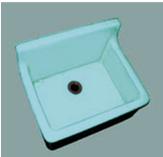
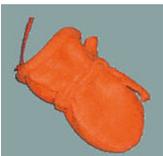
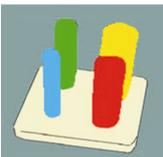
| | | |
|---|--|--|
|  | Familiar Meaningful Realistic F-M-R |  |
|  | Familiar Meaningful Non-Realistic F-M-NR |  |
|  | Familiar Non-Meaningful Realistic F-NM-R |  |
|  | Familiar Non-Meaningful Non-Realistic F-NM-NR |  |
|  | UnFamiliar Realistic UF-R |  |
|  | UnFamiliar Non-Realistic UF-NR |  |
|  | UnFamiliar Abstract shapes UF-A |  |

Figure 6.1. Examples of stimuli used in each of the seven different categories.

The subjects were given a piece of carrot or banana when they entered the learning compartment and after they had completed their session. On each trial one reward could be obtained, consisting of a pea or piece of corn combined with either a raisin or a piece of peanut. Water was available ad libitum in both the home and test cage.

The experimenter left the room after starting the task for a subject. A video camera mounted above the learning compartment allowed the experimenter to monitor the behaviour of the subjects from an adjacent room. All animals of the group were extensively trained in the separation and testing procedure before testing began, to ensure that the animals would enter the learning compartment voluntarily. In the final part of the training period all subjects were given a pre-test discrimination task, which served to familiarise them with the discrimination paradigm (i.e. choose between two stimuli, only one of which provides a reward). In this pre-test animals were required to discriminate between a blue hexagonal shape and red oval. A correction paradigm was used such that after a wrong choice the chosen stimulus would disappear only to immediately reappear on the screen. The animal could now only proceed to the next trial after pressing the correct choice. Only trials in which the first response of the animal was correct counted toward the criterion of 18 correct responses out of 20 consecutive trials.

Stimuli

Twenty-one stimulus pairs were created. The stimulus pairs varied in particular features or combinations of features (see below). Seven distinct categories of stimuli were created. For each of these seven categories three different discrimination pairs were made. Table 6.1 gives a description of the categories and the types of stimuli used in each category, Figure 6.1 shows examples of stimulus pairs in each category.

Stimuli were created to address the aims of this study. First, this work examined whether stimuli depicting 'familiar' (F, e.g. an apple or a detail of the home cage) objects were more easily discriminated than stimuli depicting 'unfamiliar' (UF, e.g. a childrens' toy) items. Secondly, we subdivided the familiar category into stimuli that were obviously relevant or 'meaningful' (M, e.g. an apple) and items that were not likely to have any relevance to the subjects ('non-meaningful', NM, e.g. a detail of the home cage). This allowed us to investigate if discrimination between meaningful, biologically relevant items is easier than discrimination between items that are known, but which have no particular meaning to the animal. Thirdly, this study aimed to examine the effect of colourfulness and contrast on the discriminability of items. To this end, new stimuli were created from the objects described above. In these stimuli, the colouration of the objects was adjusted to show brighter colours. We call these items 'non-realistic' (NR). Additionally, a category of stimuli was made with simple, abstract, uniformly coloured shapes (UF-A).

All stimuli, except UF-A, were created by placing the item on a 5x5 cm grey background. There was always a distance of approximately five millimetres between the object depicted in the stimulus and the edge of the background. The UF-A stimuli were made in a similar fashion, with the exception that there was no background. The UF-A stimuli were placed directly on the black screen. The UF-A category was made as an extreme form of the NR category, closely approximating colour stimuli used in our previous work (Chapter 5) and in earlier studies (e.g. Harlow, 1945; Cole, 1953; Rosenfeld & Van Hoesen, 1979).

For each discrimination task two stimuli with the same features, i.e. from the same category, were used. Care was taken to match the two stimuli of each pair for their level of contrast. This was done to avoid the use of stimulus pairs in which one stimulus has a much higher level of contrast than the other one does.

Discrimination test

The experiment consisted of 21 two-choice discrimination learning tasks, each with a unique pair of stimuli. In each of these tasks subjects had to learn which of the two stimuli was consistently associated with a reward. The rewarded stimulus appeared on the left or right side of a black screen in a pseudo random order, and no more than three consecutive trials on the same side. Subjects were tested on two sessions a day (see above); a session ended when: 1) the subject had received a maximum of fifty trials; or 2) a maximum time of twenty minutes had passed, 3) the subject had made 251 choices on a particular task; or 4) the subject had reached a pre-set learning criterion of eighteen correct choices out of twenty consecutive trials.

In the latter two cases, the following session for that subject would start with the next discrimination task with two new stimuli. The subjects' choice on the first trial of each task determined which of the two stimuli was designated as the correct stimulus. The stimulus that was not chosen by the subject on this first trial would be correct for the remainder of that task for that subject. This was done to avoid the situation in which subjects quickly reach the learning criterion due to a strong preference for a stimulus.

Four different orders in which the 21 tasks were presented to a subject were made. Two subjects were assigned to each presentation order, Table 6.2 gives an example of one presentation order.

Measures

The performance of subjects was measured in two different ways. First, the number of trials an animal needed to reach the pre-set learning criterion on a certain task (trials without a response were omitted) is referred to as the *trials to criterion* on that particular task. Due to large intra- and inter-individual variation in performance, a logarithmic transformation was applied to these values. The transformed trials to criterion measure did not differ from a normal distribution.

Table 6.2. Example of performance and task order for one subject

| Days | Two daily sessions: max 50 trials each | Trials | Criterion reached within session? | Task-category ¹ | Order: task number within category |
|------|---|---------|--------------------------------------|----------------------------|------------------------------------|
| 1 | 1 | 1-50 | No | UF-A (a) | 1 |
| | 2 | 51-74 | Yes | | |
| 2 | 1 | 1-50 | No | F-M-R (a) | 1 |
| | 2 | 51-63 | Yes | | |
| 3 | 1 | 1-50 | No | F-NM-NR (a) | 1 |
| | 2 | 51-71 | Yes | | |
| 4 | 1 | 1-36 | Yes | UF-R (a) | 1 |
| | 2 | 1-31 | Yes | F-M-NR (a) | 1 |
| 5 | 1 | 1-45 | Yes | F-NM-R (a) | 1 |
| | 2 | 1-50 | No | UF-NR (a) | 1 |
| 6 | 1 | 51-97 | Yes | F-M-R (b) | 2 |
| | 2 | 1-28 | Yes | F-NM-NR (b) | 2 |
| 7 | 1 | 1-50 | No | UF-R (b) | 2 |
| | 2 | 51-89 | Yes | UF-A (c) | 2 |
| 8 | 1 | 1-40 | Yes | F-M-NR (b) | 2 |
| | 2 | 1-40 | Yes | | |
| 9 | 1 | 1-50 | No | | |
| | 2 | 51-100 | No | | |
| 10 | 1 | 101-119 | Yes | | |
| | 2 | 1-50 | No | F-NM-R (b) | 2 |
| 11 | 1 | 51-70 | Yes | UF-NR (b) | 2 |
| | 2 | 1-44 | Yes | F-M-R (c) | 3 |
| 12 | 1 | 1-44 | Yes | F-NM-NR (c) | 3 |
| | 2 | 1-50 | No | | |
| 13 | 1 | 51-80 | Yes | UF-R (c) | 3 |
| | 2 | 1-20 | Yes | F-M-NR (c) | 3 |
| 14 | 1 | 1-25 | Yes | F-NM-R (c) | 3 |
| | 2 | 1-34 | Yes | UF-NR (c) | 3 |
| 15 | 1 | 1-21 | Yes | UF-A (b) | 3 |
| | 2 | 1-21 | Yes | | |

¹ See Table 6.1 for an explanation of the task categories

The learning process was also examined in more detail, using a method described by Hayes (1953; see also Lai et al., 1995; Voytko, 1999). Working backwards from the moment the learning criterion was attained, the trials were divided into blocks of 20 trials, which is the same number of trials the learning criterion was based on. For each block the percentage of correct trials was calculated. Three *learning stages* were defined according to this percentage. The boundaries of the learning stages were such that: 1) in learning stage one (LS1) subjects performed below chance level (35% correct or less); 2) the second learning stage (LS2) indicated performance at chance level (between 35% and 65% correct); 3) in learning stage three (LS3) 65% or more of the trials was correct. For each learning stage, we calculated the number of 20 trial-blocks for each task and for each subject, see Figure 6.2 for two examples. For this study LS2 is of special interest. If subjects have difficulty discriminating between the stimuli of a particular task, the number of blocks of chance performance (i.e. LS2) will be large.

Finally, we analysed the average response latency for each task. For each task we averaged the time taken by the subject from the onset of a trial to the response. This measurement will be used to examine if subjects need more time to make a choice on some tasks than on others, even if performance measures do not differ.

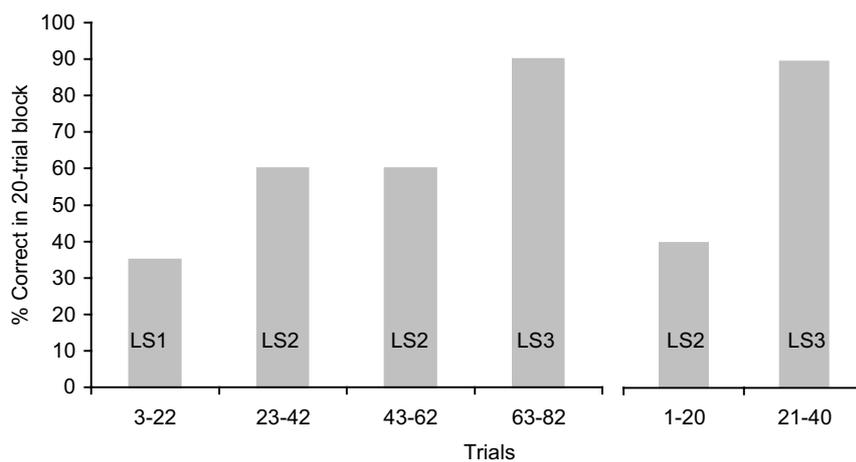


Figure 6.2. Two examples of the distribution of learning stages over consecutive 20-trial blocks. In the left example the subject reached the learning criterion in 82 trials. In the right example the learning criterion was reached in 40 trials. LS1: Learning stage 1; LS2: Learning stage 2; LS3: Learning stage 3

Table 6.3. F-, and P-values of General Linear Models for repeated measures testing within-subject effects of stimulus categories

| Do these categories differ? | Independent variables | D.F. | Dependent variables | | | | | | | | | | | |
|-----------------------------|----------------------------------|---------|---------------------|--------------|------------------|--------------------|------------------|--------------------------|------------------------------|--------------------|------------------|--------------|------------------------------------|---|
| | | | Trials to criterion | | LS1 ² | | LS2 ² | | Number of 10-trial blocks in | | LS3 ² | | Average response lat. ³ | |
| | | | F | P | F | P | F | P | F | P | F | P | F | P |
| Model I | F-M*; F-NM*; UF* (excl. UF-A) | (2, 14) | 0.11 | > 0.1 | 0.37 | > 0.1 | 0.31 | > 0.1 | 0.01 | > 0.1 ⁷ | 3.02 | 0.081 | | |
| | *R; *NR | (1, 7) | 0.00 | > 0.1 | 0.03 | > 0.1 | 0.80 | > 0.1 | 1.13 | > 0.1 | 4.37 | 0.075 | | |
| | 1; 2; 3 ¹ | (2, 14) | 8.23 | 0.004 | 0.89 | > 0.1 | 5.80 | 0.037⁵ | 5.03 | 0.023 | 1.55 | > 0.1 | | |
| Model II | F-M-R; F-NM-R; UF-R; UF-A | (3, 21) | 1.35 | > 0.1 | 0.48 | > 0.1 | 1.05 | > 0.1 | 0.39 | > 0.1 ⁸ | 1.03 | > 0.1 | | |
| | 1; 2; 3 ¹ | (2, 14) | 8.06 | 0.005 | 0.96 | > 0.1 | 5.24 | 0.046⁶ | 2.65 | > 0.1 | 2.12 | > 0.1 | | |
| Model III | F-M-NR; F-NM-NR; UF-NR; UF-A | (3, 21) | 1.23 | > 0.1 | 0.85 | > 0.1 | 0.76 | > 0.1 | 1.02 | > 0.1 | 1.46 | > 0.1 | | |
| | 1; 2; 3 ¹ | (2, 14) | 8.12 | 0.005 | 1.91 | > 0.1 ⁴ | 8.10 | 0.005 | 4.95 | 0.024 | 0.81 | > 0.1 | | |

Tests of within-subjects effects, with 'Sphericity assumed' unless otherwise noted. P-values of less than 0.1 are indicated in bold

¹ Order of tasks within each category

² Number of 20-trial blocks in learning stages 1, 2, and 3 respectively

³ Average response latency

⁴ Mauchly's test of sphericity: p = 0.015. Therefore Greenhouse-Geisser values are reported, F(1.14, 7.99).

⁵ Mauchly's test of sphericity: p = 0.036. Therefore Greenhouse-Geisser values are reported, F(1.20, 8.38).

⁶ Mauchly's test of sphericity: p = 0.036. Therefore Greenhouse-Geisser values are reported, F(1.20, 8.34).

⁷ Mauchly's test of sphericity: p = 0.042. Therefore Greenhouse-Geisser values are reported, F(1.21, 8.47).

⁸ Mauchly's test of sphericity: p = 0.010. Therefore Greenhouse-Geisser values are reported, F(1.27, 8.90).

Analyses

The goal of this study was to investigate the effect of three stimulus parameters on the discriminability of pairs of stimuli, namely: familiarity (i.e. familiar vs. unfamiliar), relevance (i.e. meaningful vs. non-meaningful), and colourfulness (i.e. realistic vs. non-realistic). The experiment yielded 21 data points for each subject. Obviously, data points of one subject cannot be considered to be independent of one another, therefore, we tested within-subject effects with a repeated measures design in all statistical tests.

Besides the three stimulus parameters mentioned above, two further aspects had to be taken into account. First, a learning effect could be anticipated across the 21 tasks, such that the third task of each category is likely to yield a better performance than the first. We defined an 'order' factor as denominating whether a task was the first, second or third within its category. This factor was incorporated in our analysis. The last aspect of our dataset that had to be taken into account in the analysis is the fact that the UF-A category cannot be subdivided into realistic (R) and non-realistic (NR) categories. To overcome this, three separate General Linear Models for repeated measures were designed.

In our first statistical test we ignored the data pertaining the UF-A tasks. We used a General Linear Model for repeated measures (Model I) to test whether there was a difference in trials to criterion between the F-M, F-NM, and UF categories while controlling for the colourfulness of the stimuli. Also, this test was used to examine if there was a difference in trials to criterion between the realistic tasks (R) and the non-realistic ones (NR), while controlling for the familiarity or meaning. Finally, the order effect was tested with this model, while controlling for the effects of the categories.

Next, we split the dataset in two in order to compare the results of the UF-A tasks with the other categories in two separate analyses. In one (Model II), we compared the results for all the realistic categories with those of UF-A. In the other (Model III), we compared the results for all the colourful, non-realistic categories with those of UF-A. In both these GLMs the order of the tasks was incorporated as in Model I.

These procedures were also used to examine the number of 20-trial blocks in the three learning stages, and the average response latency.

RESULTS

A General Linear Model for repeated measures (Model I) was performed to examine whether the feature aspects familiarity (F vs. UF), meaningfulness (M vs. NM) and the colourfulness (R vs. NR) of stimuli affected the number of trials to criterion needed by the subjects. No differences between these stimulus characteristics were found (Table 6.3 and Figure 6.3).

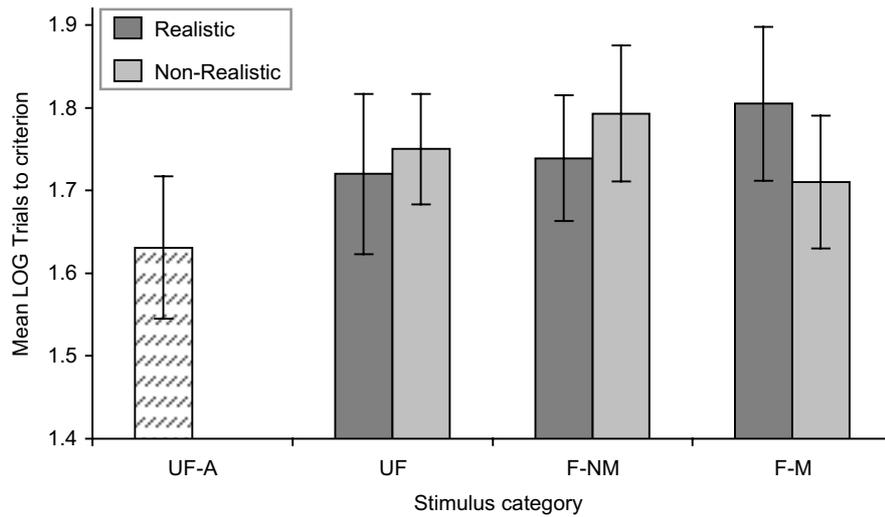


Figure 6.3. The average number of trials to criterion (\pm SEM) on tasks with different stimulus pairs, of the following categories: UF-A: UnFamiliar abstract shapes; UF: UnFamiliar; F-NM: Familiar, non-meaningful; F-M: Familiar, meaningful (see text for details).

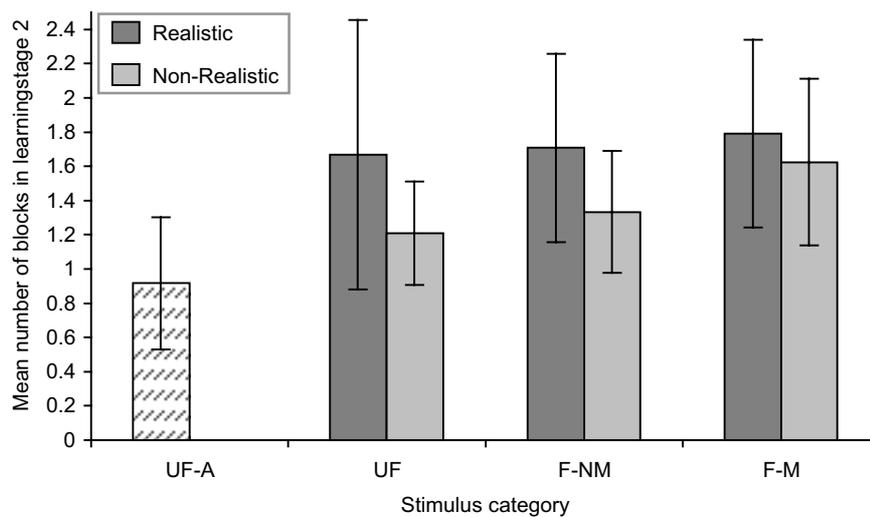


Figure 6.4. The average number of 20-trial blocks (\pm SEM) for which the subjects performed at chance level (Learningstage 2) on tasks with different stimulus pairs, of the following categories: UF-A: UnFamiliar abstract shapes; UF: UnFamiliar; F-NM: Familiar, non-meaningful; F-M: Familiar, meaningful (see text for details).

In order to include the UF-A category in the analysis two separate GLMs were run. One (Model II) tested for differences between UF-A and all other realistic categories. The other (Model III), tested for differences between UF-A and all other non-realistic categories. Neither one yielded any significant differences between the stimulus categories (Table 6.3 and Figure 6.3).

Together, these results suggest that the differences between the stimuli used in this experiment do not influence their discriminability. In other words, subjects need similar numbers of trials to reach the learning criterion irrespective of the stimuli.

The subjects may have had stronger preferences for certain stimuli in one category than in another. For instance, if subjects strongly favoured apples over bananas this may have affected the results. The analysis of the number of 20-trial blocks in learning stage one shows that there were no such differences in stimulus preference.

Similar non-significant findings were obtained for the number of 20 trial-blocks for the other two learning stages (Table 6.3). If subjects had difficulty discriminating between certain stimuli, this would result in a relatively high number of blocks in learning stage two (i.e. at chance level). The fact that no such difference was found (Figure 6.4) supports the results obtained for the trials to criterion. Likewise, no differences were revealed for learning stage three.

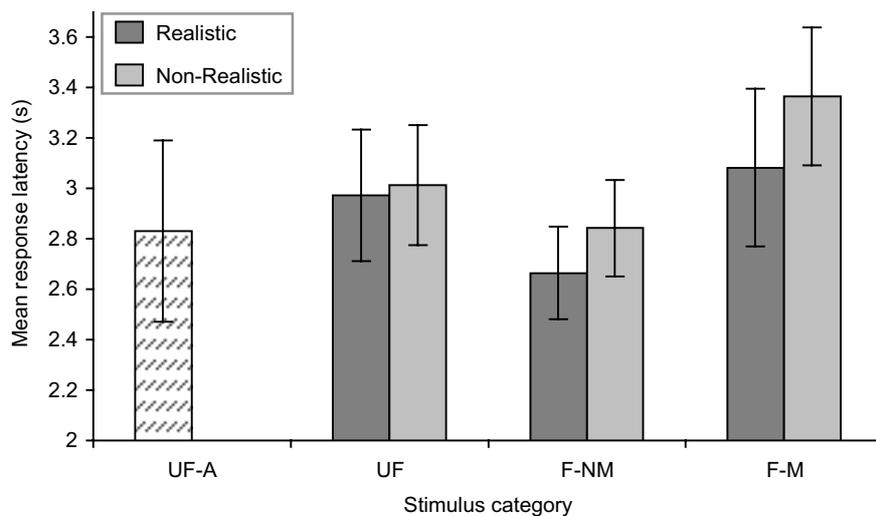


Figure 6.5. The average response latency (\pm SEM) on tasks with different stimulus pairs, of the following categories: UF-A: UnFamiliar abstract shapes; UF: UnFamiliar; F-NM: Familiar, non-meaningful; F-M: Familiar, meaningful (see text for details).

For both the analysis of the trials to criterion and the number of blocks in the learning stages two and three significant effects of task order were found. That is to say, subjects required fewer trials to reach the learning criterion when they had finished more tasks. This shows that the animals improved their performance in relation to the experience they had with the tasks. Furthermore, it indicates 'learning set' formation in the subjects (Levine, 1959; Itoh et al., 2001). There were no significant interaction effects between this learning effect and the stimulus features.

Finally, the average response time was also not significantly different between the investigated stimulus features (Table 6.3 and Figure 6.5).

DISCUSSION

This study was aimed at investigating effects of familiarity, relevance and colourfulness on the discriminability of stimuli in a discrimination test. The results suggest that familiar items do not require fewer trials to be associated with a food reward than non-familiar items. Also, meaningful familiar items do not seem to be more easily discriminated than non-meaningful familiar items. Likewise, realistic items were not associated more easily with a reward than items with brighter colours and more contrast. So, none of the stimulus attributes in this investigation significantly influenced performance.

Several studies using visual discrimination tests to examine the learning abilities of primates have yielded divergent results for the performance of subjects (Bartus et al., 1979; Moss et al., 1988; Voytko, 1999). Various methodological differences between these experiments, such as differences in stimulus features, experimental set-ups and procedures could have affected the variation in results. The present results suggest that this variation in results is not due to differences in stimulus features.

The work described here does not support the hypothesis that biological relevance improves discrimination performance. This is in agreement with the only other paper directly comparing discrimination performance on meaningful versus non-meaningful stimuli. Rosenfeld and van Hoesen (1979) reported that the initial discrimination of faces of conspecifics required a similar number of trials as the discrimination of shapes and patterns. Thus, our results also contradict the report by Dittrich (1994). However, as mentioned before, the findings of Dittrich may have been due to differences in task difficulty (Dittrich, 1988, 1994). On the other hand, the possible threatening aspect of Rosenfeld & Van Hoesen's pictures (1979) may have confounded their results (cf. Kyes et al., 1992). Furthermore, for the work described here one could argue that the effect of meaningful stimuli is more likely to occur for socially relevant pictures considering the importance of the social environment for monkeys such as long-tailed macaques ('social intelligence hypothesis' Humphrey, 1976; Silk, 1999). Future work may resolve this issue. The lack of significant differences in our results is discussed further below.

The reported importance of colour for discrimination performance (Harlow, 1945; Cole, 1953; Warren, 1954; Jarrard & Moise, 1971; Bartus et al., 1979; Rosenfeld & Van Hoesen, 1979) was not confirmed by our results. Nor do our results suggest that the reported positive effect of high levels of contrast for human recognition memory (Wichmann et al., 2002; Kornowski & Petersik, 2003) transfers to discrimination learning. Due to methodological differences a useful comparison of results can only be made with the study by Rosenfeld and Van Hoesen (1979). They reported that four experimentally naïve rhesus macaques (*Macaca mulatta*) needed on average approximately 120 trials to reach a pre-set learning criterion when discriminating between two colours (see their Figure on p. 507). In contrast, their animals needed between three hundred and four hundred trials on the discriminations of patterns and the facial discrimination. In the present study subjects needed on average 56 trials to reach the learning criterion in the geometrical shapes category. Furthermore, the highest average number of trials, i.e. 83, was found for the F-M-R category (familiar, meaningful, realistic).

A number of methodological differences may be related to this difference. For instance, the learning criterion was set higher in the Rosenfeld and Van Hoesen study, namely two out of three days with 90 % correct on 30 trials. Also, the fact that their subjects were transported to the test apparatus could have affected the performance of their subjects. Housing and rearing conditions may also affect performance of subjects in cognitive tests, but Rosenfeld and Van Hoesen do not provide details on this. Finally, the fact that our subjects had prior experimental experience does not provide a convincing explanation for the obtained difference, as the performance of the one naïve subject in this study did not differ from the others (data not shown).

The lack of significant differences in the present data may be explained by the fact that all stimuli, except the abstract shapes, were placed on a grey, square background. Thus most of our stimuli had the same outer-contour lines. Such outer-contour lines were the most dominant feature used by chimpanzees (*Pan troglodytes*) and humans in a visual matching to sample test (Tomonaga & Matsuzawa, 1992). On the other hand, in Rosenfeld & Van Hoesen's (1979) study there is also no clear difference between stimuli with different contour lines (faces) and with the same square contour lines (patterns).

An alternative explanation is that the long-tailed macaques in this study did not recognise the items depicted in the familiar stimuli. It was assumed that the monkeys would be able to recognise the objects depicted in the (familiar) stimuli based on the literature supporting this notion (for reviews see: Bovet & Vauclair, 2000; Fagot, 2000). The experiment described here was not intended to determine whether the subjects would recognise the objects shown, and the data do not allow any conclusions to be drawn in that respect. On the other hand, the fact that no differences were found does not imply that the objects were not recognised. Rather, it means that the information or meaning conveyed in these

stimuli (whether it was recognised or not) did not affect the speed of the discrimination learning. Nevertheless, the most parsimonious explanation for our results is that subjects made their discriminations purely based on physical differences between stimuli (such as shape and colour, Fagot et al., 2000). Possibly, the appearance of the depicted objects differed too much from reality. Or, perhaps, the previous experience subjects had with objects depicted in two-dimensional stimuli (i.e. the food objects of Chapter 5) was not sufficient to allow recognition in the present experiment (cf. Bovet & Vauclair, 2000; Fagot et al., 2000).

Additional studies are needed to examine whether animals can recognise the objects depicted in this study, and if so whether they would confuse them with real objects. Fagot and colleagues (2000) suggested that the recognition of objects depicted in pictures may occur at different levels of abstraction. For instance, if animals reach out to grab the depicted object, this indicates that the object was recognised, but not that the picture is recognised as a representation of the object. Rather, in such a case the animal confuses the representation with the real object. Also, extended studies are needed to clarify the conditions (such as level of contrast) that could facilitate the recognition of objects in pictures.

Finally, the results of our previous work (Chapter 5) provide an alternative way to look at the present findings. In Chapter 5 we examined performance on four reversals of a four-choice Discrimination-Reversal test. Two of the four pictures that were simultaneously presented in that test depicted food items, the other two pictures showed abstract shapes of several colours. An analysis of the correct and incorrect choices made in that experiment revealed that the abstract stimuli were more easily discriminated than the food stimuli.

One of the proposed explanations for that result was a generalisation effect. Subjects may have classified both food stimuli as 'food' rather than distinguishing between them. The present results argue against this explanation as it would predict differences between the meaningful and the unfamiliar stimuli used here.

We also suggested that the obtained differences between food and abstract stimuli could be due to physical stimulus features such as colourfulness. The food stimuli were both placed on a square, grey background and had a relatively low level of contrast compared to the abstract stimuli. The abstract stimuli were more like the UF-A category used here. With respect to the present study this suggestion would predict a difference between the realistic (R) and non-realistic (NR) stimuli, and especially between the stimulus categories most like the stimuli used in the previous Chapter 5, i.e. F-M-R and UF-A. No difference in performance was revealed between the R and NR categories. Furthermore, a post-hoc comparison between the stimulus categories F-M-R and UF-A did not provide any significant differences (performance: $F(1, 7) = 2.46$, $p > 0.1$ Greenhouse-Geisser; blocks in learning-stage two: $F(1, 7) = 2.44$, $p > 0.1$ Greenhouse-Geisser; response latency: $F(1, 7) = 1.10$, $p > 0.1$ Greenhouse-Geisser). So, neither suggestion concerning the results of Chapter 5 was supported here, possibly, due to two methodological differences between these studies.

In the previous study multiple reversals of one initial discrimination had to be made. This may have resulted in a situation in which earlier learned stimulus-reward associations interfered with the learning of later associations. Such a possible effect of 'proactive interference' (Wright et al., 1986) was absent in the present study, where new stimuli were used for each discrimination. Additionally, the previous study involved a discrimination between four, rather than two, stimuli of two different kinds. Both these methodological differences may have increased the difficulty of the previous test, making differences in stimulus features more likely to influence the results. In other words, the discrimination test as it was used here, may have been too easy to be influenced by the stimulus features we examined.

CONCLUSION

Meaning conveyed in two-dimensional stimuli does not affect performance of monkeys in a visual two-choice discrimination test. Furthermore, neither bright colours, nor enhanced contrast influence such discrimination performance. These results suggest that variation between studies using two-dimensional visual stimuli in discrimination tests cannot be explained based on differences in meaningfulness, familiarity or colourfulness of the used stimuli. This study also shows that picture recognition in primates requires more research dealing with the ways in which pictures are perceived and recognised.

ACKNOWLEDGEMENTS

We thank Martijn Wuister for assisting in the data collection and analysis. Henk Westland and Annet Louwse provided practical assistance. Han de Vries gave valuable statistical advice. We also thank Jim Anderson and Josep Call for their helpful comments and suggestions on an earlier version of this paper.

CHAPTER 7

SUMMARY AND GENERAL DISCUSSION

In this thesis, two approaches have been described to refine the primate model of human age-related cognitive decline. The influence of social aspects and temperament on cognitive functioning is the main focus of the work described in Chapters 2, 3 and 4. In Chapters 5 and 6 the importance of stimulus features as determinants of cognitive performance was examined.

First, the aims, methods and main results of Chapters 2, 3 and 4 will be summarised, followed by an integrated discussion of the findings. Next, Chapters 5 and 6 will be summarised and discussed. General directions for future research and a final conclusion will close both this chapter and this thesis.

LONG-TERM SUBORDINATION AND IMMEDIATE FACTORS AS DETERMINANTS OF COGNITIVE PERFORMANCE IN SOCIALLY HOUSED MONKEYS

Summary

Long-term factors affecting age-related cognitive decline: the importance of social history

Most primates, like humans, live in complex and hierarchical social environments. The influence of the social environment on age-related cognitive decline was the initial focus of this project. Prolonged exposure to elevated levels of glucocorticoids, associated with social subordination in female long-tailed macaques (Abbott et al., 2003; see Chapters 1 and 2 for additional references), is known to have a detrimental effect on brain structures involved in learning and memory (Belanoff et al., 2001). Moreover, a previous study in our laboratory provided initial results linking the rate of cognitive decline to a history of subordination (Veenema, 1998). In Chapter 2, the hypothesis that a combination of old age and a life time of social subordination results in relative poor cognitive capacity as compared to a combination of old age and a history of social dominance was tested.

Two stable social groups of long-tailed macaques (*Macaca fascicularis*) were used. The dominance of females of this species depends primarily on the size of their family and may remain stable for many years (Walters & Seyfarth, 1987). We used a Discrimination-Reversal (DR) test with four stimuli and multiple reversals to examine the reversal learning ability and the perseverative tendency of subjects. Subjects were extensively trained on the test procedures. As a result subjects would voluntarily enter a learning compartment to do the DR-test. In the learning compartment auditory, olfactory and limited visual contact with the group remained possible. Fifteen female subjects, ranging in age from five to 27 years, were tested. Four subjects aged fourteen or more were considered 'old'; six had previous experimental experience (Veenema, 1998). Subjects were categorised as having a history of high, middle or low dominance position.

In concordance with the literature (e.g. Itoh et al., 2001) old subjects performed poorly as compared to young subjects. Also, we found that, in accordance with the expectations, experienced subjects performed significantly better than experimentally naïve subjects did.

However, in contrast to previous results obtained in our laboratory (Veenema, 1998), the hypothesised effect of life time subordination on age-related cognitive decline could not be confirmed.

Several possible explanations can be forwarded to explain the absence of an effect of a life time of social subordination on age-related cognitive decline.

First, the proposed hypothesis may be based on incorrect assumptions. The impact of prolonged periods of elevated levels of glucocorticoids on cognitive functioning may be overestimated. Also, there may be no direct relation between subordination and elevated stress levels. However, both these aspects of the hypothesis are well founded on abundant scientific evidence. The detrimental effects of prolonged exposure to elevated levels of glucocorticoids have repeatedly been shown (e.g. De Kloet et al., 1999; Lyons et al., 2000b; Belanoff et al., 2001). Similarly, a recent meta-analysis has confirmed that in stable groups of long-tailed macaques female subordination is generally associated with elevated basal levels of glucocorticoids (Abbott et al., 2003).

Another possibility is that we could not confirm the hypothesis because the stress associated with subordination was not sufficiently intense in our subjects (but see Sapolsky, 1994; Mendoza et al., 2000). It could also be that the stress associated with subordination was not chronic. The social group may have given adequate support to subordinates to compensate the experienced stress, or the chronic nature of the stress. Von Frijtag and co-workers (2000) reported that the negative effects of social defeat in rats were attenuated in animals that were socially housed after defeat as compared to rats that were isolated following defeat. Although the support of a social group may, clearly, attenuate stress as compared to social isolation, human studies have shown that differences in social support were related to stress and the rate of cognitive decline (Arbuckle et al., 1992; Antonucci et al., 2000; Seeman et al., 2001; Zunzunegui et al., 2003). In primates subordinates generally receive more unpredictable aggression and have fewer social interactions than dominants (Melnick & Pearl, 1987; Sapolsky, 1994; Shively et al., 1997; Shively, 1998). These reports combined with the fact that in long-tailed macaques subordinate families are smaller than dominant families reduces the likelihood of the explanation that adequate social support mitigated social stress in our subjects.

The explanations forwarded above dealt with the relation between stress, subordination and cognitive performance. The following explanations will deal with methodological issues and possible confounding factors.

The chosen test paradigm is one methodological aspect that requires consideration. We used a discrimination test with four simultaneously presented

stimuli to reduce the number of rewards that could be gained by choosing in a random fashion. In combination with our finding that two of the stimuli were difficult to discriminate (see Chapter 5), this may, however, have made our DR-test too difficult to allow a differentiating effect of subordination in the old subjects. This explanation is supported by a comparison of our results with those of Veenema (1998), that revealed that our subjects needed more trials to complete the reversals.

A further difference between our study and that of Veenema (1998) could also be related to the contrasting results. For some families we tested only one or two females, rather than one female of each age category, i.e. young - middle aged - old, within each family. This may have reduced the possibility to control for idiosyncratic differences between families such as differences in temperamental aspects. Furthermore, in our study the two oldest subjects, both 25 or more years old, had a history of high dominance rank, and there was a larger age difference with the other two 'old' subjects than in Veenema's study. Moreover, these younger old subjects, 18 and 16 years of age, were perhaps too young to show a difference in the detrimental effects of prolonged exposure to elevated levels of glucocorticoids.

Finally, the hypothesis is based on the assumption that variation between young subjects was relatively small and increased as subjects grew old. This assumption was confirmed by the results of Veenema (1998). However, in our study we found large inter-individual variation also in young animals. This variation could be related to the observation that the test procedure appeared to induce anxiety in subjects, despite an extensive training period of up to ten months. This led to the suggestion that the predicted effect was overruled by other factors, such as acute stress, affecting the performance of the animals. Therefore, an investigation of several factors that could be at play here was carried out.

Immediate factors as determinants of cognitive performance independent of age

Arousal, attention and dominance

Arnsten and Goldman-Rakic (1998) demonstrated a negative effect of acute stress, that is stress experienced at or shortly before the test, on performance in primates. The test procedures used in our study were expected to elicit acute stress in the subjects (cf. Higley & Suomi, 1989; Lyons et al., 2000b), which was expected to impair performance. Bunnell and colleagues (Bunnell et al., 1980; Bunnell & Perkins, 1980) reported that a high dominance status of male long-tailed macaques was negatively related to their performance. They suggested that this was due to the concern of maintaining a dominant position while isolated from the group. Yet, from their papers it remained uncertain whether this effect was due to an increased level of acute stress or a reduced motivation to engage in the task.

In Chapter 3, we tested hypotheses about behavioural measures indicative of acute stress and motivation to engage in the task in relation to dominance status and performance in the DR-test. In addition, we predicted that experienced subjects would show less acute stress than naïve animals. The behavioural arousal during and the attention for the task were used as measures for acute stress and motivation, respectively. By doing so, these immediate factors could be assessed without interfering with the test procedures. We formulated the following hypotheses (see Chapters 1 and 3 for additional references).

Arousal

- 1) High levels of arousal are related to reduced performance.
- 2) High dominance status is related to high levels of arousal.
- 3) Experienced subjects will show relatively low levels of arousal.

Attention

- 4) Low levels of attention are related to reduced performance.
- 5) High dominance status is related to low levels of attention.

Two distinct behavioural measures were used to separate the arousal of subjects from their attention for the task. We took the percentage of time subjects visually attended to the task as an indication of their motivation to engage in it (cf. Tomporowski & Tinsley, 1996). The observed rate of displacement behaviours was taken as a general indication of the subjects' emotional arousal (Maestripieri et al., 1992; Troisi, 2002) during the test. Clearly, the aspecific alertness to (changes in) the surroundings of the individual that generally accompanies arousal is different from our measure of task oriented attention. Moreover, the behavioural arousal and attention for the task did not correlate with each other.

In contrast to our expectation, behavioural arousal had no effect on performance, nor was it related to the dominance status of subjects. Previous experience was related to high levels of arousal, which was against our expectation. In combination with the previous result that experience leads to improved performance, this finding suggests that the underlying basis of this behavioural arousal is different from the stress and state anxiety with which it is generally associated (Maestripieri et al., 1992; Troisi, 2002). It is likely, that in the present context these behaviours were not associated with anxiety and negative affect in relation to the test procedures, but rather with a positive, task related excitement (cf. Lang, 1995; 'energetic arousal' Thayer, 1996; 'anticipatory excitement' Spruijt et al., 2001; Merali et al., 2004). This possibility is supported by the result of a post-hoc analysis, which showed that a high level of behavioural arousal was associated with a high level of attention for the task (Thayer, 1996; Spruijt et al., 2001).

The present data does not allow us to determine whether the previously found association between experience and performance is mediated by arousal, or whether this is a direct relation.

With respect to the factor attention the results confirm our expectations: a low level of attention was associated with relatively poor cognitive performance. Furthermore, subjects with a high present dominance status attended less to the task. These results suggest that the negative effect of a high dominance status on cognitive performance reported by Bunnell et al. (Bunnell et al., 1980; Bunnell & Perkins, 1980) is due to a reduced attention for the task. In other words subjects with a high dominance status have a lower level of attention and thus have a reduced performance in comparison to more subordinate animals.

Trait anxiety

Individual differences in trait anxiety (Mueller, 1992) have been shown to affect cognitive performance in humans (Kumari & Corr, 1998; Li et al., 2004), primates (Schneider et al., 1991) and rodents (Ohl et al., 2002; Ohl et al., 2003). In our effort to understand the variance in performance and spontaneous behaviour of subjects during our DR-test the next step was to assess individual differences in trait anxiety in a separate experiment (Chapter 4). It is likely that subjects show different reactions in response to the testing procedures depending on their individual trait anxiety. The measure of anxiety of this separate experiment was, therefore, expected to be related to a measure of anxiety in the context of the DR-test, namely the willingness to enter the learning compartment. Also, high trait anxiety subjects were predicted to show a low level of attention for the task and poor performance. Finally, in view of the previous finding that our measure of behavioural arousal indicates a positive, task related arousal, it was expected that high trait anxiety subjects would show relatively little positive excitement for the task. In sum, we formulated the following hypotheses concerning the effects of the immediate factors trait anxiety, behavioural arousal, and attention for the task (see Chapters 1 and 4 for additional references).

- 1) High trait anxiety subjects will be relatively reluctant to enter the learning compartment.
- 2) High trait anxiety is related to reduced performance.
- 3) High trait anxiety is related to low levels of attention for the task.
- 4) High trait anxiety is related to low levels of behavioural, task related, arousal.

Subjects were repeatedly exposed to a loud noise while in the normal social group situation. We scored their spontaneous behavioural reaction to this noise. A factor analysis was used to extract principal factors from six variables quantifying the behavioural reaction of the subjects. This factor analysis yielded two factors indicating different aspects of the subjects' trait anxiety. One factor was associated with the latency to the first self-grooming bout and the latency to leave the location the animal has fled to. The other indicated the level of activity (e.g.

running, walking, and the rate of behavioural changes) of subjects in response to the noise. In the following no distinction will be made between these two factors.

In line with our predictions, high scores on individual anxiety in response to the loud noise were associated with reduced willingness to enter the DR-test compartment. This shows that the latter measure of anxiety indeed indicates the trait anxiety of the subjects.

Also in concordance with the expectations, high trait anxiety was related to reduced performance and low levels of attention for the task. These results are in agreement with previous reports on the relation between trait anxiety and cognitive performance in humans, primates, and rodents (Schneider et al., 1991; Mueller, 1992; Kumari & Corr, 1998; Ohl et al., 2003).

In line with our previous suggestion that, in the present context, our measure of behavioural arousal indicates positive excitement for the task, we found that high levels of trait anxiety were related to low levels of arousal, but only in experimentally naïve subjects. In experienced subjects arousal remained relatively high regardless of the level of trait anxiety of the animals. As experienced animals were more familiar with the test procedures and the task than naïve animals, the excitement (i.e. arousal) of experienced animals was probably not subdued by anxiety. In contrast, naïve subjects with high trait anxiety were anxious about the procedures, which counteracted their positive excitement for the task.

Discussion: dominance, attention, arousal and experience

The initial hypothesis of an accelerating effect of a life time of subordination on age-related cognitive decline was not supported by the studies presented here (Chapter 2). However, a number of other factors were found to influence cognitive performance. Results indicate that the present dominance status has a negative impact on the level of attention of these subjects which leads to a reduced cognitive performance (Chapter 3). The impact of the present dominance status may have confounded our data with respect to the initial hypothesis. The present dominance status may have increased the variation in performance among young monkeys, while it reduced the 'benefit' that old monkeys with a history of social dominance had over old monkeys with a subordinate history. In line with suggestions made by Bunnell and co-workers (1980) we proposed that dominant animals may be less motivated to attend to the task due to the 'concern' of maintaining a high dominance position, while isolated from the group. In Chapter 3 we provide preliminary evidence suggesting that 'disturbances', such as fights in the social group, divert the attention of dominant animals to the group. They may be more motivated to intervene, to protect and re-establish their rank, than to attend to the task. Additionally, the fact that present dominance status was not related to trait anxiety (Chapter 4) shows that the relation between dominance and attention for the task (Chapter 3) cannot be due to higher levels of anxiety in the dominants.

Many authors have viewed behavioural indications of arousal, such as scratching, as expressions of anxiety or acute stress (cf. 'trait anxiety' Mueller, 1992) experienced by the subject (Maestripieri et al., 1992; Schino et al., 1996; Troisi, 2002). The present results suggest that in the context of a cognitive test, behavioural arousal is associated with excitement in relation to the task, rather than anxiety and negative affect in response to test procedures. This is not to say that the more traditional view is wrong. Our results confirm that a general behavioural indication of arousal, as was used here, can be the result of different underlying emotional states. These states can be positive, i.e. anticipatory excitement (Spruijt et al., 2001), energetic arousal (Thayer, 1996), or negative, i.e. anxiety, tense arousal (see also Lang, 1995).

The previous experimental experience of our subjects had a large impact on the results of our studies. As the number of old subjects was limited, some of the subjects had previously been tested by Veenema (1998), using similar procedures. This experience resulted in improved performance as compared to naïve animals (cf. Novak et al., 1991). Furthermore, it buffered the subjects against the 'emotional strain' of the testing procedures (such as approaching the experimenter, isolation). Whereas, highly anxious naïve subjects showed a lower level of behavioural arousal than less anxious naïve subjects did, the arousal of experienced subjects was high regardless of their trait anxiety (Chapter 4). So, for experienced subjects the anxiety provoking aspects of the procedure seemed to have become irrelevant, even for high trait anxiety animals. Unfortunately, the present data do not allow a determination of whether the difference in performance between experienced and naïve subjects is a direct effect, i.e. due to a learning advantage, or an effect of higher behavioural arousal, i.e. due to an 'emotional' advantage.

Discussion: trait anxiety

High trait anxiety was not only associated with reduced performance, but also with low levels of attention for the task. This makes it difficult to assess whether the relation between trait anxiety and performance was mediated by a reduction in attention, or whether this was a direct relation, as proposed by McNaughton (1997).

Like subordination, trait anxiety may also lead to prolonged exposure to elevated levels of glucocorticoids; so one might also have predicted an interaction between trait anxiety and age in their effect on cognitive performance. This would mean that old, highly anxious subjects show a deficit in performance in comparison to old and less anxious subjects. Such an interaction effect would confound the data with respect to the initial hypothesis, especially because trait anxiety was not related to dominance status in our study.

However, no interaction between trait anxiety and age was revealed here. We measured trait anxiety as a response to a non-social situation, which may be

partly independent from trait anxiety in response to social challenges (Fairbanks, 2001; D'Eath & Burn, 2002). High trait anxiety for non-social situations may not yield a chronic elevation of glucocorticoids, and thus also yield no effect on age-related cognitive decline, because of the more infrequent occurrence of non-social challenges.

Still, individual differences in trait anxiety could possibly explain the increased variation (Rapp & Amaral, 1992; Peters et al., 1996; Gallagher & Rapp, 1997; Voytko, 1997) in cognitive performance between old, solitary housed animals. Line and colleagues (Line et al., 1989; 1991) have reported that solitary housed animals react strongly to routine caretaking procedures. For solitary housed animals different levels of life time exposure to elevated levels of glucocorticoids may be related to individual differences in anxiety in response to routine events. Also, individual differences in anxiety in response to isolation may result in differential stress exposure in those animals (Higley & Suomi, 1989). Yet, one must also consider that the relation between cortisol exposure and extended social isolation is still unclear (Sanchez et al., 2001).

A recent paper (Touyarot et al., 2004) provides another argument for a confounding effect of trait anxiety on the predicted interaction between life time dominance history and age. Touyarot and colleagues showed that high trait anxiety rats were more sensitive to the detrimental effects of prolonged psychosocial stress on cognitive performance than low trait anxiety conspecifics. So, individual differences in prolonged exposure to elevated glucocorticoid levels, due to differences in life time subordination, may be confounded by individual differences in the sensitivity to such psychosocial stress (see Veenema, 1998 for an anecdote). We have no information regarding the sensitivity of our subjects to the psychosocial stress of subordination (as mentioned above this can be different from the trait anxiety measured here). This is an important issue to consider in future studies.

We conclude that trait anxiety is a source of variation that should not be neglected. Future work could match experimental groups with respect to trait anxiety or experimental groups should be larger to allow more factors in the statistical design to filter out this variation. Here, the methods we used to separate individuals from the group posed practical limitations on the sample size (see Future directions).

With respect to the anxiety of our subjects, it is interesting to note that long-tailed macaques are a highly anxious species, relative to other macaques (Clarke et al., 1988; Clarke & Lindburg, 1993; Ward et al., 2003). So, a different species might be more suited for the experiments as described here, for instance the closely related rhesus monkey (*Macaca mulatta*). This species appears to be less anxious and has a social structure, with pronounced dominance asymmetries, very similar to that of long-tailed macaques (Melnick & Pearl, 1987; Thierry, 2000). These dominance asymmetries are an important prerequisite for the hypothesis posed in Chapter 2.

Discussion: some words of caution

The sample size of this study is not large enough to allow a full understanding of the relations among the factors discussed above. Also, it was not possible to test whether the interaction between life time subordination and age (Chapter 2) could still explain a significant portion of the data after correction for the effects of experience, trait anxiety, motivation and present dominance status. A larger sample size should make this possible (see also the section Future directions).

Furthermore, a word of caution is in place for the interpretation of the findings. In the Discrimination-Reversal (DR) test two types of stimuli were used: abstract stimuli and relevant, food stimuli (see Chapter 5). In Chapters 3 and 4 the data for these two types of stimuli were analysed separately for statistical reasons. Most findings were either significant for the abstract or the food reversals, but not for both. This calls for a modest interpretation of the results. On the other hand, the non-significant effect in one stimulus type was without exception in the same direction as the significant effect found in the other type. Furthermore, effects have to be larger in analyses with small sample sizes to reach significance. Therefore, the findings were discussed here, regardless of the stimulus type for which they were found.

Another point should also be mentioned concerning the statistical analysis of Chapter 3 and 4. The two types of stimuli were analysed separately, as were the two performance measures (i.e. trials to criterion and percentage of perseverative errors) and the factors of individual trait anxiety. The various factors were also analysed separately, because the limited sample size did not allow a simultaneous analysis of all factors. One could argue that some of these statistical tests should be considered as tests of the same null hypothesis, or as a family of related tests (Chandler, 1995). Multiple tests increase the risk of making a type I error. One way to deal with this is to apply a correction factor to the significance level. A Bonferroni correction could be used in combination with a higher significance level (0.10-0.15) to counteract the loss of statistical power (Chandler, 1995). On the other hand, one could also argue that arousal and motivation, for example, are separate in the conclusions drawn from them. So, therefore, they may constitute separate families of tests. Also, the small sample size in the present work reduced the likelihood of a type I error, because of the large effect size needed to reach significance. In consideration of these last two arguments, no correction procedure was applied and the level of significance was always kept at 0.05.

In this thesis we have used the average time subjects look at the touch screen as a very general measure of their attention for the task, and we have argued that this measure gives us an indication of the motivation of subjects to respond to the task. This general measure of attention for the task may be akin to

'sustained attention' (cf. Tomporowski & Tinsley, 1996). However, our behavioural measurement of attention is difficult to compare with detailed measures of attention dealing with the selection and recognition of stimuli such as sustained, selective and divided attention. Methods used in studies of such detailed measures of attention comprise, for instance, the reaction time in milliseconds in response to the short (40 ms) presentation of target stimuli, which occur randomly in a series of presentations of non-target stimuli (Matthews & Davies, 2001). Also, our measure is different from the way motivation is often operationalised, for instance the number of lever presses a animal is willing to make for a reward (Weed et al., 1999).

Evidently, such parameters of motivation or attention are not very suitable to be used in a learning test. Therefore, we have chosen our measure of attention, which comprised aspects of both attention and motivation to engage in the task. Furthermore, not all those detailed measures, e.g. for sustained or divided attention, are equally affected by age (e.g. Tomporowski & Tinsley, 1996; Hess et al., 2001; Hogan, 2003). So, we have refrained from stating predictions regarding the effect of age on our measure of attention.

Integration

Testing socially housed animals allows an examination of the influence of the social environment on cognitive performance. However, it also yields practical problems. Not only are such tests labour intensive, they also reveal large inter- and intra-individual variation, which can be seen as a problem in view of the small sample sizes generally involved in working with primates (compare for example Anderson et al., 1996). Nevertheless, the variation is of particular scientific interest since it draws attention to the variety of factors that may influence performance. This thesis provides the first systematic disentanglement of the web of factors affecting primate cognitive performance.

Figure 7.1 provides a summary of the relations revealed in this work. The initial hypothesis, which states that a history of social subordination accelerates age-related cognitive decline, was, in contrast to previous findings, not confirmed. We have proposed that the cognitive task used in the present study was too difficult to allow a differentiating effect among old subjects. Also, the sample of animals used in this study may not have been conducive in revealing the hypothesised effect (see Chapter 2). Furthermore, we have investigated factors that have an immediate and confounding effect on cognitive performance. Researchers aiming to compare cognitive capacities of various (experimental) groups of socially housed primates should consider the trait anxiety and dominance status of animals in each group in order to reduce variation within groups. Just as one would match experimental groups with respect to, for example, housing conditions. Our results also confirm that differences in experience profoundly affect subjects in their arousal and performance. Experienced

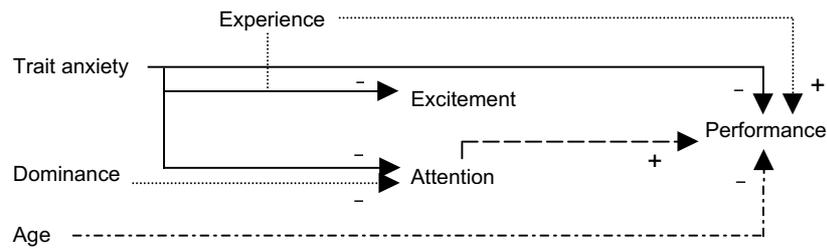


Figure 7.1. Schematic representation of the main relations found in Chapters 2-4. Arrows are printed in different styles for reasons of clarity.

monkeys are excited about the task, even those that score high on trait anxiety. On the other hand, for naïve monkeys high trait anxiety subdues their excitement. We also suggest that dominant monkeys would rather return to their group than attend to the task. The reduced attention for the task shown by dominant individuals and by monkeys with high trait anxiety impaired performance. To our knowledge motivational factors have not previously been incorporated in studies of primate cognitive performance. Most studies have used animals housed in isolation or at best in pairs. Possibly, those animals were always motivated to engage in the task as they lacked a (proper) social environment to keep them occupied (cf. Van den Berg et al., 2000). The work presented here shows that in individual tests of socially housed monkeys motivation is important to consider, especially considering the relation with dominance status. Moreover, individual differences in trait anxiety are important to consider as these may abate or amplify the long-term influence of psychosocial stress on cognitive functioning.

So, we conclude that the initial hypothesis should not be rejected based on the work presented in this thesis. We showed that the immediate factors - present dominance, trait anxiety and experience - have profound effects on both behaviour, i.e. arousal and attention for the task, and cognitive performance. These immediate factors may have obscured the predicted interaction between age and social subordination from our view. A future test of this hypothesis should control for these factors and test a larger sample of animals. In the section Future directions we propose a method to accomplish this.

There is no obvious reason why these immediate factors, excluding experience, did not have a similar confounding effect in the study of Veenema (1998). We could speculate that the more difficult test paradigm used in the present study facilitated the occurrence of these effects. Perhaps, the subjects in the easier task of Veenema's study were all equally motivated to attend to the task. Although, we aimed to improve motivation by decreasing the number of rewards

that could be obtained by responding in a random fashion, this may have had the opposite effect of bringing out differences in motivation. On the other hand, all of Veenema's subjects were experimentally naïve on his task when they were tested. So, one would expect differences in trait anxiety to influence the task related excitement of his subjects, similar to what was shown for our naïve subjects.

STIMULUS FEATURES AS DETERMINANTS OF COGNITIVE PERFORMANCE

Summary

Up to this point, we have discussed factors relating to the subjects and their social position as possible sources of variation in cognitive performance. Next, we concentrate on the effect that the nature of two-dimensional stimuli, i.e. pictures, may have on cognitive performance.

Relevance of stimuli

Based on (in)direct evidence reported in the literature on primates (Dittrich, 1994; Anderson, 1998; Fagot et al., 2000) we argued that performance on the Discrimination-Reversal (DR) test may be better for stimuli representing biologically relevant items, than for stimuli showing 'meaningless' abstract shapes (see Chapters 1 and 5 for additional references).

However, we found that stimuli depicting biologically relevant (food) items were associated with a poor performance in comparison to abstract stimuli (Chapter 5). Thorough analysis of the errors made by the subjects revealed that the used food stimuli were more difficult to distinguish from each other than the abstract stimuli. This increased difficulty was also evident in the perseverative errors after a reversal. The perseveration was stronger for the food stimuli than for the abstract stimuli. The perseverative tendency of subjects was also associated with an increase in behavioural arousal, in this case clearly due to frustration induced by the perseverative mistakes (cf. Bechara et al., 1997; Lyons et al., 2000a; Leavens et al., 2001). We suggest that the fact that the food stimuli were more similar in contrast and outer-shape than the abstract stimuli might have overruled any positive effect of the relevance of the represented items. Alternatively, a generalisation effect may have explained the results. Subjects may have classified both food stimuli as 'food' rather than distinguishing between the pictures.

Relevance, familiarity and colourfulness of stimuli

The analysis of the errors made in the reversal learning of the DR-test (Chapter 5) suggested that stimulus pairs differed in their discriminability. Therefore, a further experiment was conducted concerning the relation between stimulus features and discrimination learning (Chapter 6). Three hypotheses were formulated for this study (see Chapters 1 and 6 for additional references).

First of all, we investigated the hypothesis that stimuli depicting familiar objects are more easily discriminated than stimuli depicting unfamiliar ones. In addition, we tested whether stimuli depicting biologically relevant familiar objects improve performance more than stimuli depicting other familiar, but not relevant objects. A recent study with humans reported a comparable effect, showing that picture recognition was better for faces than for doors (Leonards et al., 2002). Finally, we hypothesised that bright and contrasting colours improved discriminability of stimuli. This hypothesis was based on the findings reported in Chapter 5 and recent work with humans showing the importance of contrast (Kornowski & Petersik, 2003).

Eight female long-tailed macaques were tested on a series of 21 discrimination tests. The stimuli of these tests varied along three variables: familiarity, biological relevance (i.e. food versus non-food items) and colourfulness (i.e. colouration and contrast). Additionally, one type of stimuli depicted evenly and bright coloured geometrical shapes. These last stimuli were comparable to the abstract stimuli used in Chapter 5 and in other studies (e.g. Harlow, 1945; Rosenfeld & Van Hoesen, 1979).

No significant differences were found between any of the stimulus traits. Thus, none of the hypotheses were supported. Familiar stimuli did not improve performance in comparison to abstract stimuli. Nor did biologically relevant familiar stimuli improve performance as compared to irrelevant familiar stimuli. This is in agreement with the only other paper directly comparing discrimination performance on meaningful versus non-meaningful stimuli (Rosenfeld & Van Hoesen, 1979). Yet, one could argue that the effect of meaningful stimuli is more likely to occur for socially relevant pictures considering the importance of the social environment for highly social animals such as long-tailed macaques ('social intelligence hypothesis' Humphrey, 1976; Silk, 1999).

Also, no effect of colourfulness was found, which appears to be in contrast to previous reports concerning colour and contrast (Fobes & King, 1982; Kornowski & Petersik, 2003). A closer comparison with the work of Rosenfeld and Van Hoesen (1979) revealed that performance of our subjects was better on all stimuli types. The fact that the learning criterion was set higher in the study of Rosenfeld & Van Hoesen than in our study, may explain this difference. The lack of differences in the present results could be related to the outer-contourlines of the pictures (a square for most stimuli). The work of Tomonaga and Matsuzawa (1992) suggests that the outer-contourlines are an important feature in the discrimination of pictures.

Discussion & integration

In Chapters 5 and 6 we examined whether features of stimulus pairs affect discrimination performance. We assumed that the objects depicted in the stimuli would be recognised by the subjects. We showed that relevant stimuli were diffi-

cult to distinguish as compared to abstract stimuli in the reversal learning of Chapter 5, while no difference in performance between stimulus types was found in the discrimination experiment of Chapter 6. The most parsimonious explanation for these results is that the subjects made their discriminations purely on the basis of physical differences between stimuli (such as shape and colour, Fagot et al., 2000). Possibly, the pictures differed too much from reality. Or, perhaps, subjects did not have sufficient previous experience with objects depicted in two-dimensional stimuli to allow recognition in the present experiments (Bovet & Vauclair, 2000). Additional studies are needed to examine whether these animals can recognise the objects depicted in this study and if so, whether they would confuse the picture with the real object as was suggested by Fagot and colleagues ('confusion mode of picture processing', Fagot et al. 2000). The latter case is indicated when an animal reaches out to grab the depicted object. Also, extended studies are required to elucidate the conditions (such as level of contrast and task difficulty, see below) that could facilitate the recognition of objects in pictures.

The errors made in the reversal phase of the DR-test, which were examined in Chapter 5, revealed that the pictures depicting relevant familiar items (food) were more difficult to discriminate than the abstract stimuli. Two suggestions were forwarded to explain this unexpected finding.

The two relevant stimuli may have been generalised as 'food' or the physical similarity of the two relevant stimuli may have made these stimuli less distinctive. In Chapter 6 these suggestions were tested by comparing discrimination performance for stimulus pairs differing in familiarity, relevance and colourfulness. No support for either suggestion was found.

Taken together, the findings suggest that proactive interference (Wright et al., 1986) played a role in the relatively poor performance for the food stimuli, on the reversals of the DR-test.

The DR-test, with four stimuli and multiple reversals, was overall more difficult than the discrimination test used in Chapter 6. This may explain why we did find a difference in performance between stimulus types in the DR-test, but not in the discrimination test. The interaction between various methodological aspects (e.g. type and number of stimuli, learning criterion) could be a topic for future studies. Likewise, the examination of circumstances in which biological relevance of stimuli can affect performance, if it does at all, is still an issue that needs to be resolved.

A recent study with human subjects reported no interaction between age and relevance of the stimuli (Leonards et al., 2002). Similarly, we found that the difference in performance between the abstract and the food stimuli in the DR-test did not depend on the age of the subjects (Chapter 5). Yet, based on our study it is too early to say whether the effect of features of the used stimuli will interact with age or the immediate factors studied in Chapters 2, 3 and 4. For instance, one could speculate that the effect on performance of the salience of stimuli depends on the level of attention of the subject.

In Chapter 5 we report that behavioural arousal in the DR-test was related to frustration occurring in tasks with a high percentage of perseverative errors. This within-subjects effect is in line with the previous suggestion (Chapter 3 and 4) that our measure of behavioural arousal was an expression of excitement in relation to the task, sometimes called anticipatory excitement (Spruijt et al., 2001), or energetic arousal (Thayer, 1996). Not receiving an expected reward may have elevated such positive energetic arousal. Clearly, the excitement a task elicits is partly due to the uncertainty of the outcome. For comparison, humans show a 'skin conductance response', a physiological measure of arousal, in a decision making task when the outcome of their choices is uncertain, but not when it is predictable (Bechara et al., 1997; Critchley et al., 2001).

To recapitulate, Chapters 5 and 6 describe our work concerning the impact of stimulus features such as relevance and colourfulness. Such features are an important aspect of the design of tests of cognitive capacities; yet little is known about their effect on performance. We demonstrated that in simple discrimination tests stimulus features, such as biological relevance, familiarity and colourfulness, do not influence performance. In more difficult tests, such as reversal learning, stimuli with bright contrasting colours and different outer-contourlines may improve performance.

The difference in discrimination performance for the stimulus types in the DR-test was not dependent on the age or dominance history of the subjects. The other immediate factors studied in this thesis also did not relate to performance on the two stimulus types. So, the food stimulus type made the DR-test more difficult for the subjects. However, this difficulty did not have a confounding effect with respect to the hypothesis that a life time of subordination accelerates age-related cognitive decline.

FUTURE DIRECTIONS

Testing socially housed monkeys adds valuable aspects to the study of cognition, but this thesis also shows that this is a difficult enterprise.

Social housing of primates allows us to study cognitive functioning within the context of the animals' natural social environment. Here, for example, we examined the proposed effect of social subordination on age-related cognitive decline. Also, cognitive functioning in relation to dominance could be compared in primate species differing in their social system. Another example could be the study of the knowledge individuals have of (kin-)relations between other monkeys (Dasser, 1987, 1988).

Limitations are encountered in the confounding influence that dominance status and differences in trait anxiety exert on the level of attention for the task and performance. Additionally, small sample sizes, generally involved in studies of primate cognition (cf. Bunnell et al., 1980; Anderson et al., 1996), hamper statistical analyses.

Future studies should aim to test a larger population of monkeys in order to be able to test for possible factors affecting primate cognitive functioning, e.g. experience, dominance status and trait anxiety. The experimental method used here is not appropriate for such a large-scale examination. It is very time consuming and does not allow tests of a substantial portion of animals in a group.

We propose a test facility that is part of the animals' home cage and that is an integrated part of their daily life and feeding routine. Such a facility should have several identical units. These units should allow monkeys to 'earn' their daily food rations by performing in various tests. These units should also allow monkeys to be tested individually, without the intervention of an experimenter. If monkeys cannot temporarily separate themselves from the group then dominance relations are likely to inhibit subordinates from performing (Drea, 1998; Drea & Wallen, 1999). Such a test facility would provide a means to test all monkeys in a population (or at least a substantial sample from all age and rank classes). Moreover, it has the advantage that all animals will (gradually) become experienced with it. Moreover, new-born animals start their habituation to it at an early age. So, in time the benefit of experience shown here may be bestowed on many, if not all subjects.

Applying such a method to a large sample of primates would make it possible to reveal the full extent and intricacy of the multitude of factors influencing primate cognitive functioning. This would be a valuable contribution to the understanding and use of primates as models for human cognitive functioning. Furthermore, it would allow new research concerning, for instance, the relevance of the social system for the cognitive capacities of primates. These and other questions could be addressed using a variety of different tests, which could be applied with relative ease.

Also, such a system makes it possible to assess longitudinal questions of cognitive development and decline, since Washburn and co-workers (2001) reported that monkeys will retain their motivation to perform in such systems over long periods of time.

Finally, more research is needed to study the way in which pictures are perceived and recognised by primates. More work is also needed to determine whether, and in what circumstances biologically relevant stimuli can improve (discrimination) learning. One important aspect to consider in this respect is whether pictures show socially relevant stimuli such as faces of group members (cf. Humphrey, 1976 'social intelligence hypothesis'; Dittrich, 1994). For studies aiming to investigate the knowledge primates have of (kin-)relations between others (Dasser, 1987, 1988) it is especially important to know whether subjects recognise the individual monkey depicted in two-dimensional stimuli. The methodology proposed above can facilitate tests of picture recognition in monkeys.

CONCLUSION

This thesis addresses the possibilities and limitations of individual tests of cognitive performance of socially housed primates. Possibilities lie in the relation between the social environment and individual cognitive performance. The initial aim of this project was to confirm the previously reported accelerating effect of a life time of social subordination on age-related cognitive decline. The present data did not support the results of that previous study. However, several other effects, namely limitations related to testing socially housed monkeys, may have obscured the predicted effect.

This thesis provides the first systematic evaluation of the multitude of psychosocial risk factors to affect primate cognitive performance. The central role of low attention as an indication of motivation, which was related to high dominance status on the one hand and reduced performance on the other, is especially noteworthy and has not previously been established in primates.

Stimulus features are an important methodological aspect to consider in the design of a cognitive test, provided that the overall difficulty of the task is sufficient to allow a differentiating effect. In difficult tests stimuli with bright, contrasting colours and different shapes are easily discriminated; biologically relevant stimuli do not appear to improve performance further.

In summary, this thesis highlights the importance of the social environment for primate cognitive performance and it gives an insight into the complicated interactions between the individual and its social life. Furthermore, successful cognitive performance on tests may depend on the brightness and contrast of the applied stimuli. Thus, this thesis may guide students of cognitive capacities of social animals in the design of their studies.

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SAMENVATTING

Over het algemeen genomen verslechteren de cognitieve vermogens van volwassen mensen. Deze leeftijdsgerelateerde achteruitgang is echter niet voor iedereen even sterk. De variabiliteit in de resultaten van studies naar de cognitieve vermogens van mensen neemt toe naarmate de gemiddelde leeftijd van de bestudeerde groepen hoger is. Er zijn vele factoren gesuggereerd die deze toename in variabiliteit zouden kunnen verklaren. Een belangrijke factor betreft de langdurige blootstelling aan verhoogde concentraties glucocorticoïden. Deze stoffen, waaronder cortisol, spelen een belangrijke rol in de stressrespons. Deze stressrespons is de fysiologische reactie van het lichaam op onveilige, onzekere, oncontroleerbare en bedreigende situaties. Echter, langdurige blootstelling aan glucocorticoïden heeft een nadelig effect op cognitieve vermogens van mensen en dieren. Bij dieren is langdurige blootstelling aan glucocorticoïden in verband gebracht met sociale factoren. In recent onderzoek bij mensen is een vergelijkbare suggestie gedaan. Recent onderzoek toonde aan dat mensen die weinig steun uit hun sociale omgeving ondervonden relatief slecht scoorden op testen van hun cognitieve vermogens. De onderzoekers suggereerden dat dit een gevolg is van langdurige blootstelling aan glucocorticoïden. Echter, de sociale structuur van de menselijke samenleving is helaas zeer complex, hetgeen het moeilijk maakt om de levenslange invloed van de sociale omgeving op cognitieve achteruitgang te bestuderen. Primaten bieden wel de mogelijkheid dit te onderzoeken.

Primaten vertonen vergelijkbare leeftijdsgerelateerde neuro-anatomische en cognitieve veranderingen als mensen en zijn derhalve een goed model voor de menselijke cognitieve veroudering. Bovendien vertonen primaten ook een toename in de inter-individuele variatie bij het stijgen van de leeftijd. Bij diverse primatensoorten is langdurige blootstelling aan glucocorticoïden in verband gebracht met een lage sociale positie. Bovendien blijft de sociale rangorde bij sommige soorten erg stabiel. Bijvoorbeeld, de onderlinge rangorde van vrouwelijke Java-apen (*Macaca fascicularis*) blijft jarenlang gelijk. Dit maakt dat bij dergelijke dieren die in stabiele sociale groepen leven, de lange-termijn-invloed van subordiatie op cognitieve vermogens onderzocht kan worden. Oude vrouwelijke Java-apen met een geschiedenis van subordiatie zullen slechter presteren op een test van cognitieve vermogens dan oude vrouwtjes met een geschiedenis van dominantie. In eerder onderzoek is deze relatie inderdaad aangetoond, nl. bij de sociaal gehuisveste kolonie Java-apen op het Ethologie Station van de Universiteit Utrecht. Deze resultaten vormden de aanleiding voor het huidige project.

In **Hoofdstuk 2** werden de cognitieve vermogens van vijftien vrouwelijke Java-apen getest in een 'Discrimination-Reversal' (DR) leertaak, waarbij de dieren moesten leren te discrimineren tussen een aantal stimuli. De subjecten kregen voor elke poging steeds dezelfde vier plaatjes te zien op een aanrakingsgevoelig computerscherm. Eén van de stimuli werd consequent beloond. Er gold een criterium van negen goede keuzes uit tien opeenvolgende pogingen ('trials'). Wanneer een dier dit criterium had gehaald, werd de beloningsrelatie gewisseld ('reversal'). Nu werd één van de andere plaatjes beloond, tot dat het criterium

opnieuw werd behaald. De subjecten vertegenwoordigden verschillende klassen van leeftijd en ranggeschiedenis.

In het huidige onderzoek kon de hypothese dat een geschiedenis van subordination het cognitieve verouderingsproces versnelt, niet bevestigd worden. Naast theoretische overwegingen, zijn er belangrijke methodologische aspecten die aan dit resultaat ten grondslag kunnen liggen.

De leertaak van het huidige experiment was mogelijk te complex om verschillen in cognitieve prestatie tussen oude dieren aan te tonen. Ook kan het zijn dat de samenstelling van de huidige steekproef van dieren misschien minder evenwichtig was dan de steekproef uit het eerdere onderzoek. Bovendien werd in het huidige experiment niet alleen een grote inter-individuele variatie gevonden bij de oude dieren, maar ook bij de jonge dieren. Bij onderzoek naar de cognitieve vermogens van primaten zijn kleine steekproeven gebruikelijk. Inter-individuele variatie, zoals die ook hier gevonden is, kan er dan toe bijdragen dat eventuele effecten niet zichtbaar worden. In dit project zijn daarom, naast het lange termijn effect van ranggeschiedenis, een aantal factoren onderzocht die de prestatie van de dieren op een directe manier kunnen beïnvloeden, zoals acute stress, aandacht, huidige dominantie status, individuele angstigheid en ervaring.

De reactie van de dieren op de experimentele procedures kan tot verschillende niveau's van acute stress hebben geleid, met een negatief effect op de prestatie. De acute stress die een dier ervaart in reactie op de procedures zal mede afhangen van individuele verschillen in 'angstigheid'. Angstigheid kan worden gezien als een onderdeel van het temperament van een dier. Bij zowel mensen als apen zijn individuele verschillen in angstigheid gerelateerd aan verschillen in cognitieve prestaties. Ook zou de sociale positie van een dier een directe invloed kunnen hebben op de prestatie. Zo werd gevonden dat dominante mannelijke Java-ape slechter presteerden op een cognitieve taak dan subordinaten. Dit directe effect van dominantie op de prestatie kan het gevolg zijn van verhoogde acute stress bij de dominante dieren, die veroorzaakt kan zijn door verlies van controle over de groep. Ook kan een verschil in motivatie een rol hebben gespeeld. Dominante dieren waren misschien minder gemotiveerd om de test uit te voeren, bijvoorbeeld omdat ze minder gemotiveerd waren om beloningen te verdienen.

In dit project zijn de relaties tussen acute stress, motivatie, huidige dominantiestatus, angstigheid en cognitieve prestatie onderzocht. Aangezien een aantal dieren reeds ervaring met een vergelijkbare cognitieve taak had opgedaan, werd de factor 'ervaring' ook betrokken in de analyses.

In **Hoofdstuk 3** werd het gedrag van de dieren tijdens de DR-leertaak gekwantificeerd. Voor de mate van acute stress en motivatie werden gedragsmaten gedefinieerd. De frequentie van zogenaamde oversprong-gedragingen werd gehanteerd als een indicatie van de opwindings van een dier. De procedures van de cognitieve test leiden bij de dieren waarschijnlijk tot acute stress, welke zich kan uiten in dergelijk gedrag. Dit opwindingsgedrag werd daarom als maat genomen voor de acute stress. De aandacht die een dier besteedde aan de cognitieve taak werd als indicatie gezien voor de motivatie van dat dier om de taak te doen. De angstigheid van de dieren werd op twee manieren bepaald. Ten eerste

werd de bereidheid om mee te werken in de DR-leertaak als maat voor angstigheid in de context van de cognitieve testprocedures gebruikt. Ten tweede werd de angstigheid van de dieren in een apart experiment (**Hoofdstuk 4**) onderzocht. De dieren werden in de 'normale' setting van de sociale groep in de thuiskooi worden blootgesteld aan een zeer hard geluid. Aan de hand van het gedrag van de dieren in reactie op dat geluid werd de angstigheid van het dier bepaald. De relatie tussen deze twee maten werd ook onderzocht.

De resultaten geven aan dat: 1) de mate van opwinding niet is gerelateerd aan de prestatie; 2) dieren met meer aandacht voor de DR-leertaak relatief goed presteren; 3) angstige dieren relatief slecht op de DR-leertaak presteren en weinig aandacht tonen; 4) angstige dieren minder geneigd zijn om de mee te werken in de DR-leertaak 5) dominante dieren weinig aandacht tonen voor de DR-leertaak; 6) dominantie niet is gerelateerd aan de mate van opwinding; 7) angstige naïeve dieren minder opwinding tonen dan minder angstige naïeve dieren; 8) bij ervaren dieren er geen verschil is in opwinding tussen angstige en minder angstige dieren.

Deze resultaten geven aan dat onderzoekers van cognitieve capaciteiten van primaten hun experimentele groepen gelijk zouden moeten maken voor wat betreft dominantieverhoudingen en individuele angstigheid. Dominantieverhoudingen kunnen de aandacht van dieren voor de cognitieve taak beïnvloeden en daarmee de prestatie. Bovendien kunnen de lange-termijn-effecten van subordinatie verzwakt of juist versterkt worden door individuele verschillen in angstigheid.

De resultaten laten tevens zien dat de overspronggedragingen in de context van deze cognitieve test een uiting zijn van positieve opwinding gerelateerd aan de leertaak. De experimentele ervaring van sommige dieren in de DR-leertaak had een aanzienlijke invloed op het gedrag en de prestatie van deze dieren. Bij de naïeve dieren nam de opwinding voor de leertaak af als functie van hun angstigheid. Bij de ervaren dieren, daarentegen, had de angstigheid geen effect op de opwinding. Mogelijk is dit effect geleid tot het resultaat dat ervaren dieren beter presteren op de DR-leertaak dan naïeve dieren.

In het eerste deel van dit proefschrift werd het primatenmodel van cognitieve veroudering verfijnd met factoren die gerelateerd zijn aan de dieren. In het tweede deel lag de nadruk op de mogelijke effecten van stimulouseigenschappen op de prestatie in discriminatie-leertaken.

Het gebruik van twee-dimensionale stimuli (plaatjes) in testen van cognitieve vermogens heeft een aantal voordelen boven het gebruik van objecten als stimuli. Plaatjes zijn eenvoudig te creëren, te manipuleren en constant te houden binnen en tussen experimenten. Ook kunnen testprocedures geautomatiseerd worden. In toekomstige studies zal het gebruik van plaatjes derhalve belangrijker worden. In eerder werk werden diverse soorten stimuli gebruikt, zoals neutrale geometrische vormen maar ook plaatjes van dagelijkse objecten. Het is bekend dat kleur, in vergelijking met zwart-wit stimuli, een belangrijke eigenschap is die het leren van een discriminatietaak (discriminatieleren) versnelt. Echter over de invloed van andere aspecten, zoals biologische relevantie van de afgebeelde

objecten of de mate van contrast in plaatjes zijn slechts enkele studies bekend, waar bovendien geen eenduidige conclusie uit te trekken valt.

In **Hoofdstuk 5** werd de hypothese getoetst dat plaatjes van biologisch relevante objecten zoals voedsel-items makkelijker te discrimineren zijn dan plaatjes van abstracte geometrische vormen. In de hiervoor vermelde DR-leertaak werden steeds vier plaatjes aangeboden: twee voedselplaatjes en twee abstracte plaatjes. De keuzes van de dieren werden geanalyseerd in opeenvolgende wisselingen (reversals). Uit deze analyse bleek dat de voedselplaatjes juist moeilijker te discrimineren waren dan de abstracte plaatjes. Dit zou verklaard kunnen worden doordat de dieren de voedselplaatjes generaliseren als 'voedsel' zonder onderscheid te maken tussen de plaatjes onderling. Ook is het mogelijk dat de voedselplaatjes leidden tot een slechtere prestatie doordat deze uit minder felle kleuren bestonden, dat wil zeggen minder contrastrijk waren. Eerder onderzoek suggereert dat de omtrek van stimuli een belangrijk element is voor discriminatieleren. Het feit dat de voedselplaatjes een gelijke omtrek hadden kan daarom mede bepalend zijn geweest voor het gevonden verschil.

In **Hoofdstuk 6** werd het effect van stimulouseigenschappen op discriminatieleren nader onderzocht. Acht vrouwelijke Java-apen kregen een serie voorgelegd van 21 discriminatieleertaken. In elke taak werden twee stimuli met dezelfde combinatie van eigenschappen aangeboden. De taken verschilden onderling in de volgende eigenschappen van de afgebeelde objecten: bekendheid, biologische relevantie of de mate van contrast. In deze studie werd geen effect van de stimulouseigenschappen op discriminatieleren gevonden. Ook werd in deze laatste studie geen van de suggesties bevestigd die de moeilijkere discriminatie van de voedselplaatjes in de DR-leertaak zou kunnen verklaren.

In combinatie laten de resultaten van deze twee hoofdstukken zien dat in simpele twee-keuze discriminatieleertaken stimulouseigenschappen, zoals bekendheid, relevantie en contrast geen effect hebben op de prestatie. Echter in moeilijkere taken, zoals de vier-keuze DR-leertaak, hebben felle kleuren en veel contrast een positieve invloed op het discriminatieleren. Biologische relevantie heeft geen additioneel effect.

Concluderend bieden de studies van dit project een eerste systematische evaluatie van de psychosociale risicofactoren voor cognitieve prestaties van primaten. Zij benadrukken het belang van de sociale omgeving voor cognitieve prestatie van primaten en geven inzicht in de complexe relaties tussen het individu en de sociale omgeving. Van bijzonder belang en nog niet eerder beschreven, is de centrale rol van beperkte aandacht voor de leertaak, die enerzijds gerelateerd is aan een hoge dominantiepositie en anderzijds aan verminderde prestatie. De eigenschappen van stimuli vormen een belangrijk methodologisch aspect waarmee bij het ontwerpen van cognitieve taken rekening gehouden moet worden.

DANKWOORD

Als eerste wil ik natuurlijk mijn promotoren bedanken. Jan, Berry en Thea, bedankt voor jullie vertrouwen en goede adviezen. Jullie diverse achtergrond zorgde voor een gevarieerd aanbod aan meningen en suggesties, die elkaar vaak goed aanvulden. Dat ik was aangesteld bij het UMC, werkte bij de faculteit Biologie en uiteindelijk promoveerde bij de faculteit Diergeneeskunde leverde soms verwarrende situaties op, vooral voor de andere mensen van jullie instituten: 'Volwassenen psychiatrie', 'Gedragsbiologie' en 'Ethologie en welzijn'. Jullie hebben mij altijd naar die afdelingen toe gesteund, waarvoor ik jullie dankbaar ben. Nu ik het toch over die verschillende afdelingen heb, wil ik ook de mensen die zorgden voor de (secretariële) ondersteuning bedanken voor hun assistentie.

Het college van Bestuur van de Universiteit Utrecht en de Lucie Burgers Stichting voor Vergelijkend Gedragsonderzoek hebben mij financieel ondersteund tijdens de laatste fasen van dit project, waarvoor mijn dank.

Jan, jou wil niet alleen bedanken voor de afgelopen vijf jaar maar voor al je adviezen en steun sinds ik 11 jaar geleden als student voor het eerst bij je aanklopte met de mededeling dat ik de cognitie van apen wilde bestuderen.

De leerstoelgroep Gedragsbiologie was altijd mijn thuisbasis. Speciale waardering gaat dan ook uit naar Jan, Johan, Renee, Han, Liesbeth, Thijs, Sonja, Serge, Hayo, Rinus, Marie-José, Theo, Hans, Annet, Henk, Sharon, Frouke, Simon, Koos, Judith, Matthijs, Zjeffrie, Suci, Mandy, Miranda, Eleni en Brigitte voor jullie morele en organisatorische steun, de gezelligheid en goede discussies. Liesbeth, bedankt voor de goede suggesties en voor de stok achter mijn deur tijdens het schrijven.

Een aantal mensen hebben essentiële bijdragen geleverd aan dit onderzoek. Henk, Annet, Miranda, Hans en Theo hielden het Ethologie Station overeind, zorgden voor de apen en (heel belangrijk) hielden de testruimte schoon. Henk, bedankt dat je altijd klaar stond om te helpen met apen leren en apen schuiven. Ook Annet was er altijd met goede raad en kennis van de apen en ook van de Observer, waar ik dankbaar gebruik van heb gemaakt. De heren van het mechanisch en elektronisch atelier wil ik bedanken voor de opstellingen die zij met veel kennis en kunde bouwden en draaiende hielden. Rob, Rob en Gerard, ik vond het altijd zeer plezierig om met jullie samen te werken en ik heb jullie aandacht voor mij persoonlijk erg op prijs gesteld. Han, je was altijd bereid om geduldig en onvermoeibaar al mijn statistiekvragen te beantwoorden, dankjewel. En dan zijn er nog de studenten die ik mocht begeleiden: Nicolle, Michelle, Léonie I, Patricia, Leonie II, Saskia, Myrke, Martijn C., Martijn W., Dragosh, Sandra, dank aan jullie allen voor jullie tomeloze inzet en voor de goede discussies. Ik heb veel van jullie geleerd. Dank ook aan Els die, net van school, als vrijwilliger apen wilde trainen. Zonder jullie allen was dit dankwoord nooit geschreven.

Werken in de (wetenschappelijke) geïsoleerdheid van het Ethologie Station is niet altijd makkelijk, maar aan de andere kant de uitstekende sfeer (b.v. tijdens de lunch, uitstapjes, etentjes en video-avonden), de collega's en studenten maken dat ik daar met veel plezier aan terugdenk. Bedankt: Henk, Annet, Miranda,

Eleni, Patrick, Brigitte, Hans, Theo, Snow, Carla, Veronique, Morris, Geesje, Suzanne, Jaantje, Mischa, Wendy, Christella, Mikey, Eric, Marjolijn, Klaas, Joost, Boris, Nicolle, Michelle, Léonie I, Patricia, Leonie II, Saskia, Myrke, Martijn C., Martijn W., Susan, Arianne, Jerta, Suzanne, Ramon, Griet, Karlijn, Maartje, Marjolijn, Chaim. In het bijzonder ben ik Miranda, Brigitte en Griet dankbaar dat ze me bij de laatste loodjes toch, zo nu en dan, achter mijn computer vandaan sleurden voor de broodnodige ontspanning.

Eleni en Brigitte, ik ben blij dat ik lief en leed van het aio-schap met jullie kon delen, bedankt voor jullie luisterend oor en opbeurende woorden. Een woord van dank ook voor twee experts op het gebied van apen trainen en leertaken: mijn voorganger Hans en Miranda. De prettige discussies, goede adviezen en nuttige suggesties heb ik zeer gewaardeerd.

Op deze plek wil ik ook wat woorden wijden aan de apen. Ik bedank ze allemaal voor de mooie tijd en goede herinneringen die ze me hebben meegegeven. De standvastigen, Milva en Ikea, natuurlijk omdat ik altijd op ze kon rekenen. De twijfelaars, Felix, Alfa, Kraa, Kaa, Yukka en Bilboa, omdat die het elke dag spannend maakten; ondanks alle frustatie had ik ze niet willen missen. En dan zijn er natuurlijk ook nog Hoeba, Lixa, Virginia, Roza, Freya, Rastafa en Yudea die elk op hun eigen wijze in mijn herinnering gegrift staan. Maar ook de andere leden van de Roza en Lixa groep, zoals Vip, Regilio, Voodoo, Goa, Clint en Pifa, zorgden voor vreugde en ook voor frustratie. Een betere training in geduld kon ik niet krijgen.

Mijn ouders en broer wil ik bedanken voor hun interesse, voor het bedwingen van hun nieuwsgierigheid, en natuurlijk voor het vertrouwen dat ze altijd in me hebben gehad. Děkuji své české rodině a přátelům za jejich zájem, pochopení a trpělivost.

Tot slot: Dana, er zijn zoveel redenen om jou te bedanken. Dank je wel voor je hulp bij het opmaken van dit boekje. Voor alle dalen waar ik zonder jou niet uit gekomen was. Kortom: bedankt voor alles wat je de afgelopen jaren voor me hebt betekend !

CURRICULUM VITAE

Ido Bart Toxopeus was born on August 10th, 1973 in Amsterdam and grew up in Muiderberg. He spent his high school years at the Goois Lyceum in Bussum where he graduated in 1991. In the autumn of that year he started his studies Biology at the Utrecht University.

His deep-rooted interest in (primate) cognition led to an internship at the Ethology Station of the Utrecht University. Supervised by Dr. Hans Veenema he worked on a procedure to conduct cognitive tests with socially housed monkeys. For his second internship he spent a year in the United States in the laboratory of Prof. Daniel Povinelli. Under his supervision he explored the ability of chimpanzees to recognise themselves on delayed video recordings. In the final stage of his masters studies he wrote a project proposal with Professor Jan van Hooff to investigate the long-term memory of chimpanzees. He received his masters degree (doctoraal diploma) in 1997. In the following year he spent several months in Indonesia and participated in an ecological field study of sea turtles.

In March 1999 he began his PhD-studies at the medical department of the Utrecht University on an inter-departmental project concerning the social influences on age-related cognitive decline in monkeys. This research resulted in the present thesis.

Next to his PhD-work, in August 2000, Ido Toxopeus was appointed on a part-time position to set up and co-ordinate the new Primate Brain Bank. In this position which continued into 2004, he created an organisational structure for the Primate Brain Bank and co-ordinated the conservation and adjudication of primate brain material.

