THE CORPUS CALLOSUM IN THE LATERALISED REACHING TASK

AN INVESTIGATION OF THE ROLE OF THE CORPUS CALLOSUM IN THE

LATERALISED SKILLED REACHING TASK

By

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ABSTRACT

Long-term potentiation (LTP), a long-lasting enhancement of synaptic efficacy, is believed to be the mechanism by which memory storage occurs in the brain. Several studies have shown that LTP can be induced in various neural sites, not only by electrical stimulation, but also as a result of behavioural modifications. It has previously been shown that LTP in the primary motor cortex accompanies motor skill learning. One study showed that potentiation occurred following training on a lateralised skilled reaching task. In this task, animals are trained to use only one paw to grasp a small food pellet. An interesting finding that has been uncovered from these studies is that, although only one hemisphere actively participates in the task (the trained hemisphere), the other hemisphere (untrained hemisphere) also shows potentiation. This has led to the hypothesis that the corpus callosum is involved in the transfer of information from one hemisphere to another during training on the reaching task. The nature of this communication, however, is unknown. Two possibilities were considered. The first was that the callosum transfers information that allows the animal to maintain its balance while the reaching paw is elevated. Careful observation of videorecording made while animals performed the task however, failed to reveal any deficits in balance in animals that had undergone a callosal transection.

A second possibility is that the corpus callosum transfers information about the

task from the trained to the untrained hemisphere such that, even though it does not actively participate in the task, the untrained paw may "know" how to perform above chance level. Analysis of the rate of successful reaching with the untrained paw revealed no advantage for normal animals over transected animals. Work is however, currently underway to increase the number of animals in the study in order to obtain a more conclusive result.

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INTRODUCTION

The question of how we store volumes of information during the course of a lifetime has occupied memory researchers for well over a century. Earlier, philosophers were interested in knowing how we come to have memory and which organ was responsible for its storage. Aristotle, around 450 BC, postulated that memory was a property of animals that perceive time since, according to him, memory implied that some time had elapsed. He therefore concluded that "the organ whereby [we] perceive time is also that whereby [we] remember" and that organ was the soul. Later, it was accepted that the brain was the centre of the body's functional abilities and after years of discussing memory as a philosophical topic, it became a science in the 19th century.

William James in 1890 defined memory as "...the knowledge of an event, or fact, of which meantime we have not been thinking...". James recognised the contribution of associationists to his view of memory, stating that recollection of stored information occurs through the recall of ideas that are associated with the information in question. In 1885, Herman Ebbinghaus had already defined two forms of memory, one that could be voluntarily recalled and another that was reproduced involuntarily. He also recognised that our ability to store certain kinds of information depended greatly on our attention and interest while we were learning the material. For example, as pointed out by Ebbinghaus

himself, "The burnt child shuns the fire, and the dog which has been beaten runs from the whip, after a single vivid experience". Taken together, the definitions given by both James and Ebbinghaus, with a few refinements, have withstood the test of time.

Having established a general definition of memory, the next logical step was to look more closely at exactly where in the brain memory is stored and to investigate its underlying mechanisms. The search for the site of memory storage began in earnest in 1902 with the work of Shepard Franz and continued with his student Karl Lashley. These psychologists sought to find the site of memory storage through lesion studies. By systematically lesioning different areas of the brain, they hoped to hit upon one that would result in the loss of memory. Lashley however, found that the location of the lesion did not really affect memory; the size of the lesion was more important. His conclusion was that memory is diffusely stored throughout the brain. Subsequent research has shown that many areas may participate in memory formation and recall, but they do not do so equally. For example, research has shown that the hippocampus is essential for the formation of some forms of spatial memories (Bohbot et al., 2000; Moser and Moser, 1998; Wilson and McNaughton, 1993), while the amygdala is required for storage of memories for emotional events (Canli et al., 2000; Adolphs et al., 2000; Maren, 1999). The idea that memory was diffusely stored raised questions about how it is possible to connect different sensory modalities in the representation of one memory. For example, how is it possible that both the smell and the sight of apple pie can trigger a strong memory of one's grandmother? The answer to this question was in part, provided in 1949

by Donald Hebb. He proposed the theory that memory storage occurs when connections between co-active neurons become strengthened. According to his theory, the internal representation of an object is held in short-term memory as long as there is activity through the connections between the cells. If this activity persists long enough, the connections between the cells are strengthened and memory consolidation occurs. This process allows memory to be transferred from a short-term to a long-term storage site. Since it only requires the co-activity of neurons, it also allows for associations, including those across sensory modalities.

Hebb proposed a mechanism by which memories could be stored, but for several decades, researchers had no way to test it. In 1973, Bliss and Lomo, while studying synaptic transmission in corticohippocampal pathways in a rabbit, reported a phenomenon that seemed to substantiate Hebb's finding. They were investigating the effects of electrical stimulation to the perforant pathway, a bundle of axons that carries information from the cortex to the hippocampus. They found that high frequency stimulation of the perforant path produced a long-lasting enhancement of synaptic transmission in the activated pathway. This long-lasting enhancement of synaptic efficacy following high-frequency stimulation is referred to as long-term potentiation (LTP). Bliss and Lomo's results were consistent with a mechanism by which synaptic connections between co-active neural cells could be strengthened, but further research was required to verify this property. Most of this research was carried out in the hippocampus, a site that is known to be involved in memory storage (Barnes, 1995;

Staubli et al., 1995; Eichenbaum, 1995). Through these studies, some of the molecular

mechanisms that underlie LTP have been established. It is now known that most of the

LTP that is induced in the brain is dependent on the activation of N-methyl-D-aspartate

receptors (NMDARs). The discovery of these receptors not only strengthened Hebb's

theory of synaptic facilitation via co-activity of neurons, but also provided a mechanism.

NMDARs in post-synaptic neurons respond to the release, by the pre-synaptic neuron, of

the excitatory neurotransmitter glutamate. However, the NMDAR channels, which allow

calcium ions to enter the post-synaptic cell, remain closed due to a physical barrier

imposed by the presence of magnesium ions. These magnesium blocks can only be

removed if the post-synaptic cell is sufficiently depolarised. Depolarisation occurs as a

result of the entry of sodium through another set of co-activated glutaminergic receptors,

the α-amino-3-hydroxy-5-methyl-4-isoxazole propionate receptors (AMPARs).

Activation of these receptors is strictly transmitter dependent. The NMDAR has

therefore been described as a coincidence detector since both the pre-synaptic cell

(release of glutamate) and the post-synaptic cell (depolarisation) have to be active at the

same time in order for the receptor to open. This co-activity leads to synaptic

strengthening as posited by Hebb. Calcium that enters through the NMDARs facilitates a

cascade of protein kinase activity that may eventually lead to protein synthesis and

structural modification of the synapse. The demonstration that LTP satisfied the Hebb

rule was encouraging, but was not sufficient evidence that LTP is a memory mechanism.

Although Bliss and Lomo's report showed that LTP possesses some of the properties

associated with memory in the mammalian brain, several more examples have been considered and are listed below.

We are capable of learning some information after only one exposure to a stimulus. It would not, for example take multiple trials for "the burnt child" in Ebbinghaus' example to remember that fire burns! However, other forms of information storage require multiple trials, such as learning to drive a car. If different types of information require varying amounts of time to be consolidated into the memory system, and these rate differences can be attributed to the properties of the brain's memory systems, then LTP might also be expressed differently within these systems.).

Experiments involving induction of LTP have shown that it possesses these characteristics; it takes longer to induce LTP in the neocortical than in the hippocampal system. Trepel and Racine (1998) showed that LTP induction in the neocortex requires application of stimulation over a number of days whereas induction in the hippocampus can occur after one set of high frequency stimulation trains. These findings are consistent with the putative roles of the hippocampus and neocortex in memory storage as suggested by McClelland et al. (1995); the hippocampus is involved in the rapid acquisition of relational associative memory, while the neocortex shows a slower learning rate.

Once information is stored in the brain, it can remain there for very long periods of time. Most adults can remember information from as early as their fifth year of life. Of course, information may last for that long due to some form of passive rehearsal; recalling a particular event activates and strengthens its memory trace. This passive

rehearsal is akin to repeated LTP stimulation in a system over an extended period of time.
If this were done, then one would expect LTP to last throughout the animal's life, even if
it showed gradual decay following any individual session. Even without repeated
stimulation however, LTP should last long enough to reinforce its position as a putative
mechanism for memory storage.

LTP has been shown to be a relatively long-lasting phenomenon, in some cases
lasting from several weeks to several months (Barnes, 1979; Racine, 1983; Abraham,
1986). Additionally, LTP is specific to the activated pathway; neighbouring inactive
pathways remain unaffected or show a weakening of the synaptic response
(Schwartzkroin & Wester, 1975; Andersen et al., 1977; 1980; Lynch et al., 1977;
Dunwiddie & Lynch, 1978; Barrionuevo & Brown, 1983).

We have already discussed the Hebbian properties of LTP, but its associative
properties must be verified. For example, a weak input paired with a strong input should
show LTP effects not achievable by activation of the weak pathway alone.

The associative properties of LTP have been extensively studied. Simultaneous
activation of convergent pathways yields a greater LTP effect than activation of one of the
pathways in isolation (Lee 1983). Moreover, weak inputs incapable of inducing LTP
alone can generate an LTP effect if activated concurrently with activation of a separate
but converging strong pathway (Levy & Steward 1983). Associativity has now been
demonstrated in several pathways that converge in the hippocampus and it shows
properties similar to classical conditioning, which is a well-established form of

associative learning (Levy & Steward, 1983).

More evidence for the connection between LTP, learning and memory has been provided by considering electrophysiological changes that occur following learning. Sharp et al. (1985) demonstrated that exploratory behaviour in rats produces a change in magnitude in perforant path synaptic responses over the course of exploration. One interpretation is that the rats learned something about the new environment, and this learning was reflected in LTP-like changes in the perforant path. In a more recent but similar study, researchers have reported incremental increases in field excitatory post-synaptic potentials (EPSPs) during training in a radial arm maze (Mitsuno et al., 1994).

The most interesting evidence for the role of LTP in learning has come from pharmacological studies. It had to be shown that blocking LTP blocks memory or that increasing LTP increased the rate of learning. As discussed earlier, the NMDARs are critical for the induction of one form of LTP, particularly in the CA1 region of the hippocampus (Coan et al., 1987; Malenka and Nicoll, 1993). If this form of LTP is a mechanism by which memory storage occurs, then one should expect that blocking NMDARs and thus LTP, should impair learning and memory. Morris et al. (1986) showed that the performance of rats in a water maze was impaired in response to a competitive NMDAR antagonist. The rats were unable to learn when the NMDARs were blocked. The antagonist also completely blocked the induction of LTP.

The theory of LTP as a putative mechanism for memory storage has not however, been without criticism. Through several experiments, Peter Cain has argued that studies

such as the one by Morris et al. (1986) mentioned above, are incorrect in the conclusions they reach. In three studies carried out by Cain and colleagues, it was found that if rats were first familiarised with the experimental apparatus, they could perform the water maze task normally even in the presence of an NMDAR antagonist. Cain et al. concluded that NMDARs are not crucial for spatial learning in the water maze task. They argued that the water maze task consists of several components, and it is incorrect to infer that the animals are not learning due to the blockade of one population of receptors. However, in spite of these criticisms, LTP has continued to be widely accepted as a possible mechanism for memory storage. Even if one were to accept Peter Cain's arguments against LTP, and ignore the evidence from NMDAR blockade, there would still be ample instances were LTP, learning and memory could be investigated, and their connection verified.

The majority of the evidence considered thus far, has been provided by experiments that looked at LTP in the hippocampus and in the formation of spatial memory. However, LTP is not restricted to this neural structure alone. It has also been shown in the amygdala, a neural site linked to emotional memory storage (Aroniadou-Anderjaska et al., 2001) and in the auditory and visual cortices (Kudoh, 1997; Otsu,1995). Most importantly for the following study, activity-dependent modifications can also occur in the motor cortex. The primary motor cortex (M1) is a region that contains a somatotropic representation of the motor subdivisions of the body musculature (Weiss and Keller, 1994). M1 is necessary for skilled voluntary movement and it may

also play a pivotal role in motor learning. This hypothesis stems from research that has shown that M1 is capable of rapid reorganisation following motor skill learning (Karni et al., 1995; Nudo et al., 1996; Morales et al., 1999), changes in limb configurations (Sanes et al., 1992) and nerve lesions (Donoghue et al., 1990). Previously, motor learning was studied using operant conditioning (Sasaki and Gemba, 1982; Thompson, 1986; Woody, 1986). However, this method required that the animals perform simple tasks using existing reflexes and did not necessitate the learning of new skilled movement. It is the acquisition of new skilled movement that results in modification of horizontal connections in M1.

In rat M1, horizontal connections are formed by the axons of pyramidal cells in layers II - IV. Reorganisation after nerve lesions in M1, however, has been shown to occur primarily in pyramidal cells located in layer II/III horizontal fibres (Donoghue, 1997; Huntley, 1997). The results from lesion studies by Donoghue and Huntley raised the possibility that the same changes in horizontal connection efficacy may be observed after skilled motor learning. This hypothesis was tested by Rioult-Pedotti et al. (1998). Rioult-Pedotti trained rats to perform a reaching task in which they used only one preferred paw. Rioult-Pedotti looked at electrophysiological responses in the motor cortices and found that there was an increase in amplitude of the EPSPs in the horizontal connections in the hemisphere contralateral to the paw that had been trained in the reaching task (henceforth referred to as the trained hemisphere). This study was the first to show that there were changes in the cortical connections in the motor cortex after the

acquisition of a new motor skill. The changes were likely caused by mechanisms similar to those that cause changes seen during LTP induction since it was difficult to induce LTP in the trained hemisphere after skill acquisition.

The Rioult-Pedotti study provided an interesting new method for looking at the link between LTP, learning and memory. Her results have since been replicated and extended in our lab. In their original experiment, Rioult-Pedotti et al. measured cortical changes in brain slices taken from trained animals. Ji and Hodgson replicated this experiment and Hodgson and Boyd then ran a similar experiment using acute preparations. After training the animals on the reaching task, Hodgson and Boyd placed a stimulating electrode and a recording electrode in each hemisphere. In the acute preparation, the callosum, a band of fibres that connects the two hemispheres, is intact. Thus Hodgson and Boyd were able to record a response in the motor cortex of one hemisphere when they stimulated the callosum closer to the opposite hemisphere. The pathway they were testing went from layer II/III of the motor cortex in the stimulated hemisphere through the callosum to layer II/III in the opposite hemisphere. They found that when they stimulated the trained hemisphere and recorded in the untrained hemisphere (hemisphere ipsilateral to the reaching paw), the evoked potentials were greater than when they recorded in the trained hemisphere after stimulating the untrained hemisphere. I replicated this study using chronic preparations and found similar results. The untrained hemisphere showed some potentiation even though it had not been directly exposed to the skilled reaching task. One possible reason for the increased efficacy seen

in the untrained hemisphere could have been that during the reaching task, the animals may have attempted to use the non-preferred (untrained) paw. These few attempts could possibly have caused the changes seen. This however, was refuted by the study by Hodgson and Boyd in which they had mouth fed controls. These animals also attempted to reach for the pellet, although infrequently, and no facilitation of cortical connections was seen in either hemisphere. This raised the possibility that the corpus callosum was involved in the transfer of information from one hemisphere to the other.

Theoretically, in bimanual behaviour, both hemispheres of the brain are cognisant of the task being performed since both hands are participating. When the task is performed by one hand, the hemisphere that controls that hand is aware of what is being done. However, the other hemisphere may also learn about the task through transfer of information via the corpus callosum. The callosum's ability to transfer information from one hemisphere to another has also been seen in patients suffering from epilepsy. In these patients, the epileptic focus may be in one hemisphere, but the seizure activity may be transferred to the other hemisphere via the callosum. Treatment for these subjects usually involves removal of the corpus callosum. Patients who have had their callosa removed have provided interesting research subjects for verification of the role of the callosum, and studies have shown that when a task is performed by one hand, the hemisphere ipsilateral to this hand is completely unaware of what the opposite hemisphere is doing. In further studies, Hunter et al. (1975) and Thut et al. (1997) verified that an intact callosum facilitates the transfer of information from one hemisphere to another. In their

respective studies, subjects with callosal transections and normal, control subjects, were trained to perform a task using one hand. The subjects then had to use the other, untrained, hand to repeat the task. This transfer of information was reflected in the facilitation of performance on a task with the untrained hand. In both cases, subjects with callosal transections showed impairments in performing the task with the untrained hand in comparison to the control subjects.

In a study by Eacott and Gaffan (1990), the ability of monkeys to perform a visuomotor task with the untrained hand after callosal transection was examined. Some of the animals in this study underwent partial callosotomy. The results of the experiments showed that animals with complete callosal transections showed no intermanual transfer, while those with partial transections performed better. This study is important in that it showed that transfer of learning could occur when some part of the callosum was left intact, and that incomplete transections can confound the results obtained in callosal transection experiments.

The studies mentioned above strengthen the hypothesis that the callosum is involved in transferring information during the skilled reaching task. The nature of that communication, however, is unknown. One possibility is that the callosum is transferring information to the untrained hemisphere, making it aware of the change in posture during the task in order that the animal can maintain it's balance while it's reaching paw is elevated. A second possibility is that information about the reaching behaviour is being transferred from the trained to the untrained hemisphere such that, even though it does

not participate in the task, the untrained paw may "know" how to perform above chance

level. The following study examined these two possibilities.

METHOD

Subjects

Thirty-three adult, male Long-Evans hooded rats were used in this experiment. At the time of surgery, the animals weighed 250-300 g. The animals were housed separately and kept on a 12-h light/12-h dark schedule. During training, food was restricted to maintain their body weight at roughly 85% of the free-feeding weight. Water was provided ad libitum.

Surgical procedure

Two days before surgery and up to five days after, the animals were given the antibiotic Septra in their water. They were anaesthetised with sodium pentobarbital (65mg/kg) and received atropine methyl nitrate (1.2 mg/kg) to prevent respiratory distress. After the procedure, the animals were given a few drops of diluted cherry flavoured children's Tylenol. A few drops were also placed in their drinking water.

The surgery followed normal stereotaxic procedure. Callosal transection was achieved using a No. 20 scalpel blade which had the non-cutting edge ground down to within 1 mm of the cutting edge (see Figure 1). A 2 mm wide groove beginning 4.5 mm and ending 13 mm anterior to Bregma, was drilled to a depth which left a fine sheet of bone above the dura. The tip of the blade was then lowered through the dura and passed

between the olfactory bulb. The blade was passed caudally, lowered and withdrawn. The groove was filled with bone wax before suturing the skin. The animals were allowed 10 days to recover before behavioural training commenced. Sham surgical procedures were identical except the scalpel blade was not lowered once it had been inserted through the dura.

Figure 1:   **A** is the modified scalpel blade used to make the callosal transection, in

comparison to a normal scalpel, **B**. The blade was a No. 20 blade, with the

non-cutting edge ground down to within 1 mm of the cutting edge.

A

B
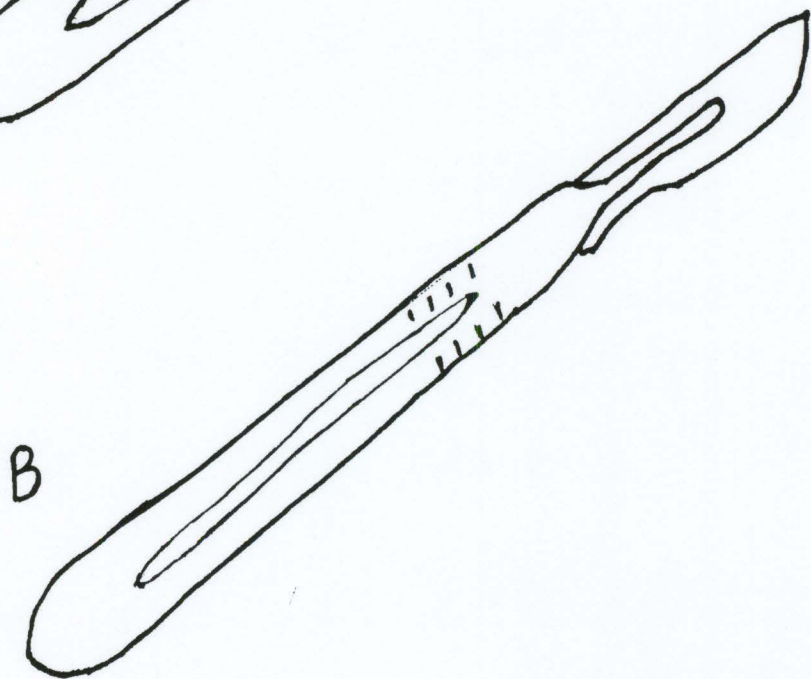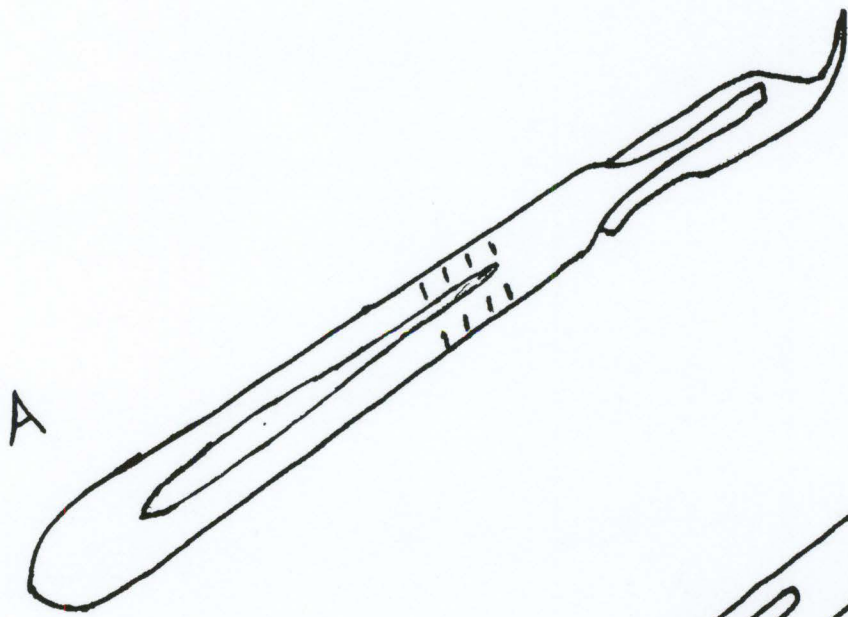
Behavioural procedure

Each animal was placed in a plexiglass reaching cage; width 16 cm, length 35 cm and height 30 cm (see Figure 2). Small food pellets (honey Rice Krispies) were placed on a platform immediately outside a 2 cm opening in the reaching cage. Rats were trained to reach through the slot and grasp the food pellet using one paw. The pellets were placed far enough away from the opening to discourage mouth feeding. The training lasted eleven days; five days for each paw, and one day in between during which the animals did not perform the reaching task. Each training session featured a pair of rats and lasted approximately 45 minutes.

Before training, the animals were separated into two groups. The first group, referred to as the free-choice reaching group, consisted of animals using their preferred paw to reach for the pellet in the first five days of the experiment and the non-preferred paw for the second 5-day block. The second group was a forced-choice reaching group. Animals in this group had to reach with their non-preferred paw during the first five days and then switch to their preferred paw. These animals were initially permitted to reach five consecutive times so that their preferred paw could be ascertained. After this time, they were trained to reach with their non-preferred paw. During the second week of training, all animals were reluctant to switch paws. They were therefore initially handed food to the correct paw and gradually the pellet was placed on the platform.

Figure 2: The cage used to train animals on the skilled reaching task. Animals were

placed inside the cage and trained to reach through a 2 cm wide slot (**A**),

for a Rice Krispie pellet placed just outside of the slot on the platform (**B**).

30 cm

35 cm

A

B

←16 cm→
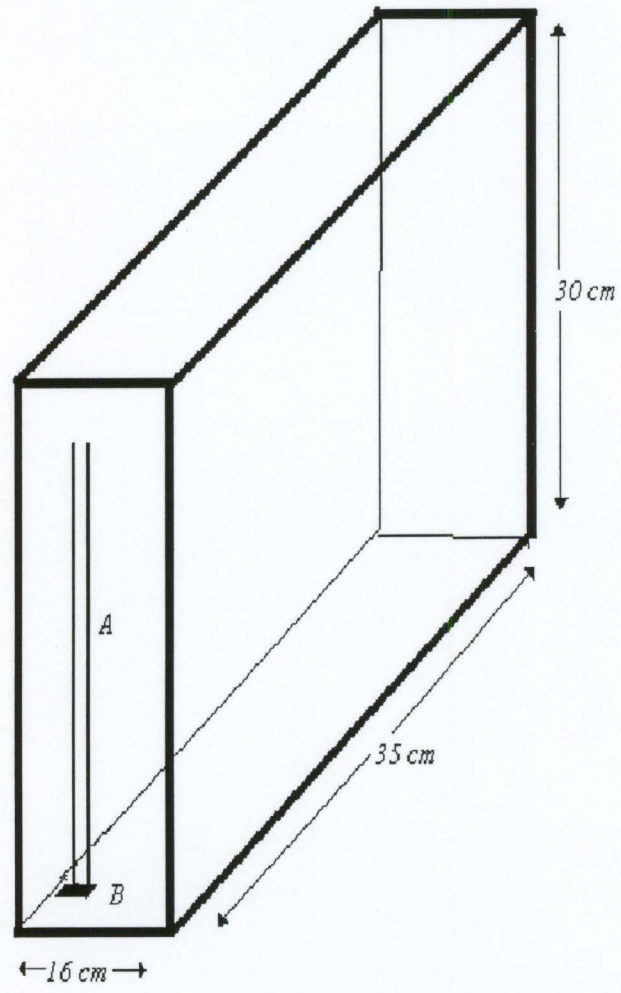
Videotaping

During some of the training sessions, some pairs of animals were videotaped for analysis of their behavioural posture. The reaching cages where numerically labelled 1 and 2 so that the animal number did not appear on the recording. In this way, judges would be unable to guess which animal belonged to which of the three groups; normal control, sham control and experimental.

Histology

At the end of the training period, the animals were injected with a large dose of urethane and perfused through the heart with phosphate buffer solution followed by buffered formal saline. The brains were serially sectioned in the coronal plane. The sections, which were 50 μm thick, were stained with cresyl violet solution and the extent of the callosal lesion was examined.

RESULTS

Histological examination allowed the animals that had undergone the surgical procedure to be more carefully differentiated into a sham and an experimental group (see Figure 3). Animals that had large lesions to the brain where excluded from data analysis (see Figure 4).

Videotape observations

Observation of the videotaped training sessions revealed that there were no noticeable differences between the experimental and control animals. Videotapes were viewed by two independent judges who were blind to the animals' group placements. Although viewing took place over two days, identical procedures were followed during both days. On each day of viewing, the judges were shown a tape of a normal control and a sham animal performing the reaching task in the first week of the experiment in order to familiarise them with the task. After this, they were asked to view three separate groups of animals; experimental and sham, experimental and normal control, and sham and normal control. In each case, judges were asked to be attentive to where and how the animal placed its non-reaching paw during training and also to the movement of the rest of the animal's body. Judges were shown from the 25th to the 30th minute of the 45 minute reaching session. During the first viewing session, judges were shown recordings

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taken on the first and third day of block one of training. During the second session, they

viewed recordings from the first and the third day of training in block two. Judges were

not alerted to the change in the animals' training blocks.

Figure 3:   Histological sections showing the three groups of animals. The sections

were 50 μm thick and stained with cresyl violet solution. Section **A** shows

a normal control animal; section **B** is from an animal that underwent a

sham surgical procedure and section **C** is a callosally transected animal.

A
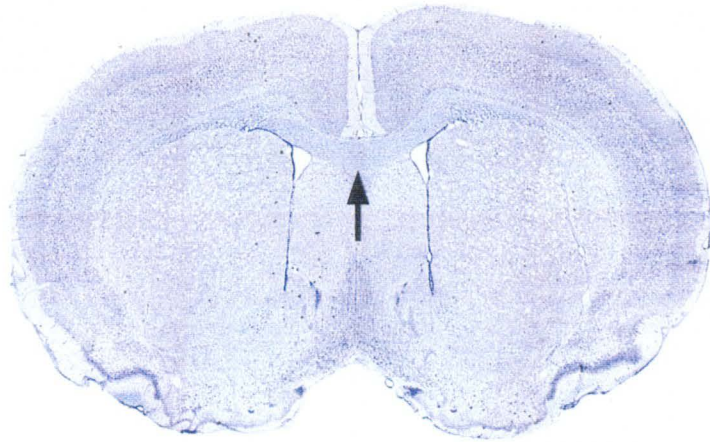
B
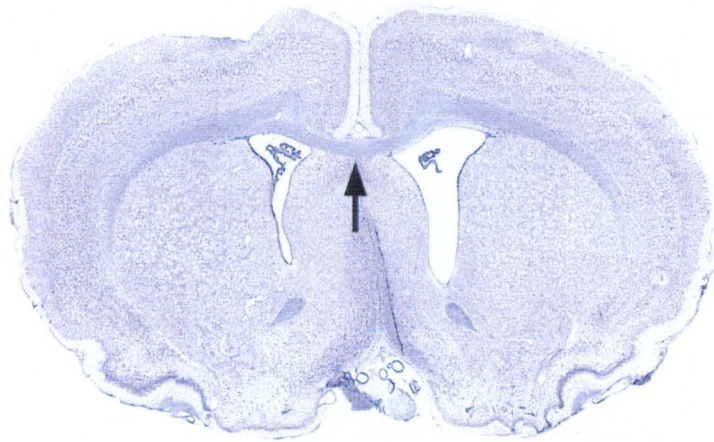
C

Figure 4:   Histological section of an animal that had a large lesion to the brain as a

result of the surgical procedure. Data from this animal and from others
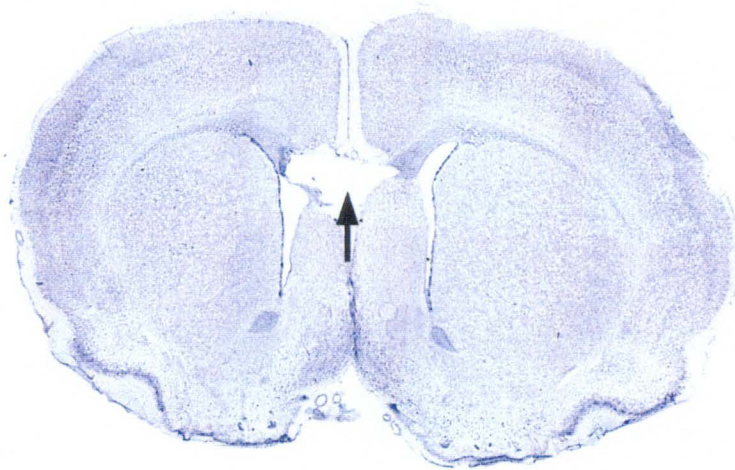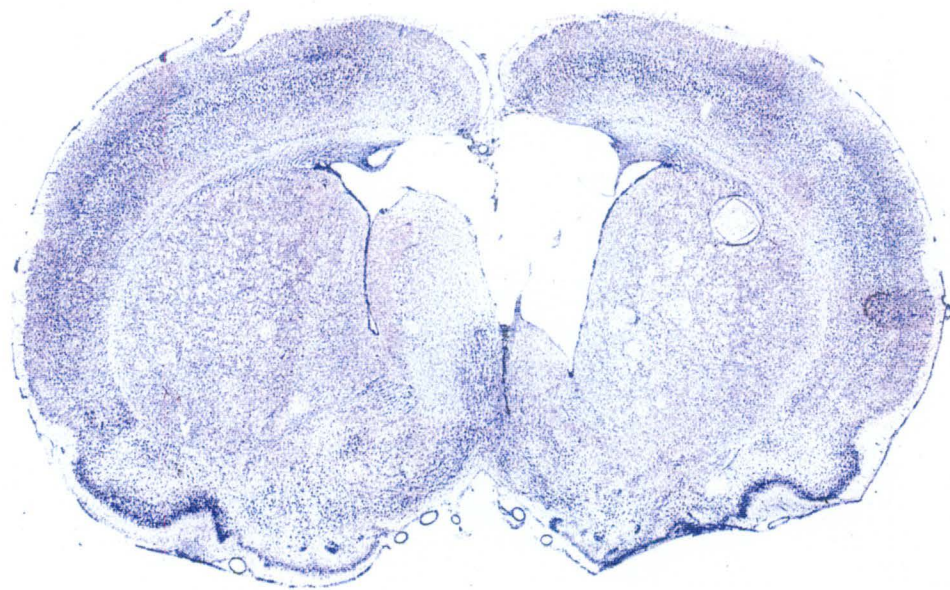
with similar lesions were excluded from analyses

Behavioural data analyses

Free-choice reaching group

The group consisted of 26 animals divided into three subgroups: experimental

(n = 9), sham (n = 9) and normal controls (n = 8)

All data were analysed using a two-way ANOVA with days as a repeated

measure. The first analysis was on the total number of reaches attempted by the animals

over both weeks of the experiment. The measures included both successful attempts i.e.

the animal managed to grasp and eat the pellet without first dropping it, and missed

attempts i.e. the animal knocked the pellet off the platform or dropped it before he could

eat it. For the trained paw there was a significant effect of day ($F(4,92) = 71.0$, p <.001

and $F(4,92) = 91.9$, p<.001 respectively), but no significant effect of group ($F(2,23) = .65$,

p =.53 and $F(2,23) = 1.13$, p =.34) and no interaction effect ($F(8,92) = .98$, p =.47 and

$F(8,92) = 1.37$, p =.22) (see Figure 5). For the untrained paw, there was a significant

effect of day, but only in week two ($F(4,92) = 22.5$, p< .001). There was no significant

effect of group and no interaction effect in either week ($F(2,23) = .04$, p =.96 and $F(2,23)$

= .65, p =.53) and ($F(8,92) = 1.04$, p =.41 and $F(8,92) = .48$; p =.87) respectively (see

Figure 6).

To see if performance improved with training, the percent correct reaches were

analysed over weeks one and two. Percent correct reaches were defined as the number of

successful reaches divided by the total number of attempts. For the trained paw, there

was a significant effect of day for both 5-day blocks ($F(4,92) = 31.0$, p < .001, and

$F(4,92) = 8.52$, p< .001, respectively). There were however no significant effects of group

in either week ($F(2,23) = 1.11$, p =.35 and $F(2,23) = 1.82$, p =.18) and no interaction

effect ($F(8,92) = 1.30$, p =.25 and $F(8,92) = 1.06$, p =.39) (see Figure 7). There were also

no significant differences for the untrained paw in either the first or second week of

training ($F(4,92) = 2.36$, p = .06, and $F(4,92) = .40$, p = .81); ($F(2,23) = .04$, p = .96, and

$F(2,23) = 1.21$, p =.32); and ($F(8,92) = 1.04$, p = .41, and $F(8,92) = .77$, p = .63),

respectively (see Figure 8).

Figure 5: The total number of reaching attempts, both successful and missed, made by the animals using their trained paw. Graph **A** shows attempts made during the first week of training, while Graph **B** shows attempts made during the second week of training after animals had switched paws. Data were collected from animals in the free-choice group.
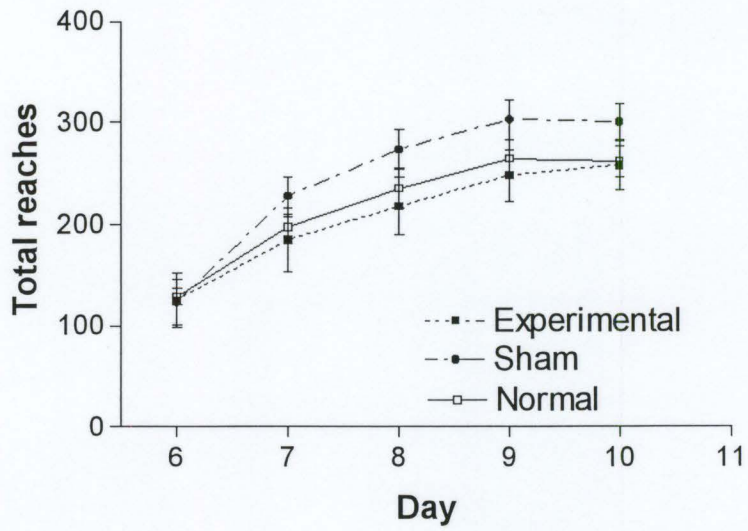
A

B

32
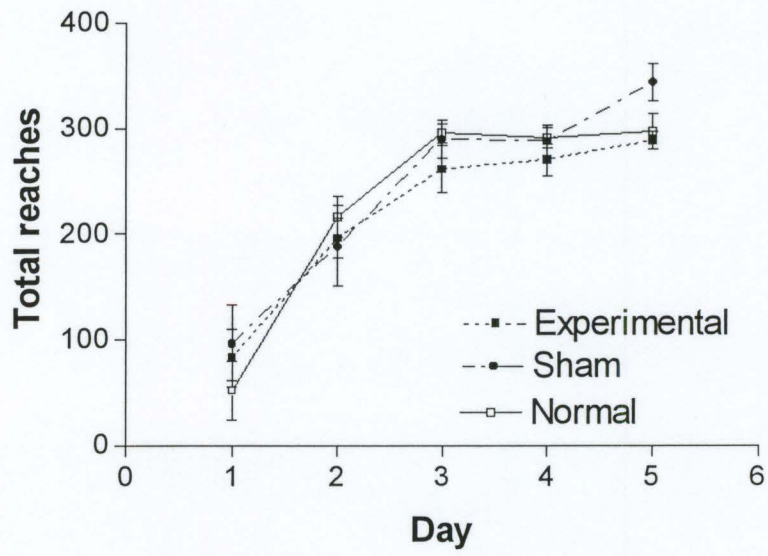
Figure 6: The total number of reaching attempts, successes and misses made with the untrained paw in both the first (graph **A**) and second (graph **B**) block of training. As training progressed in each week, the animals gradually stopped using the untrained paw. Data plotted were from animals in the free-choice group.

A
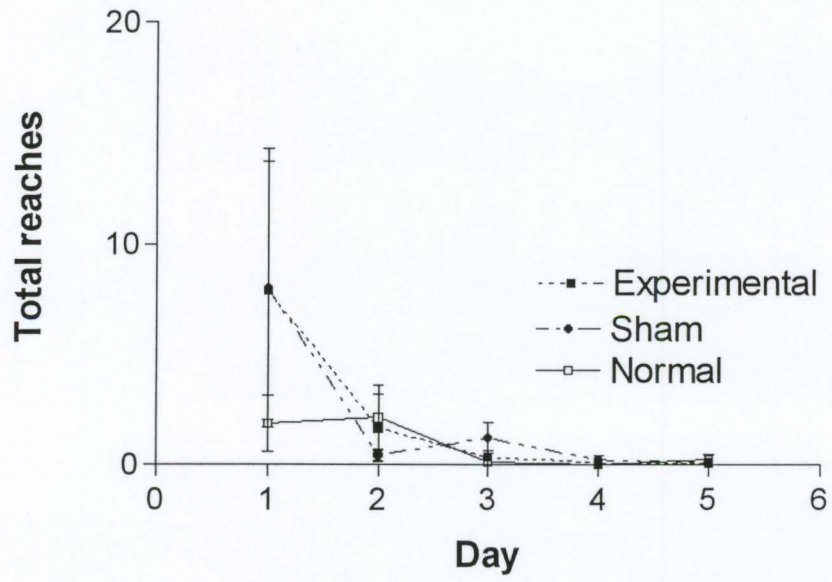
B
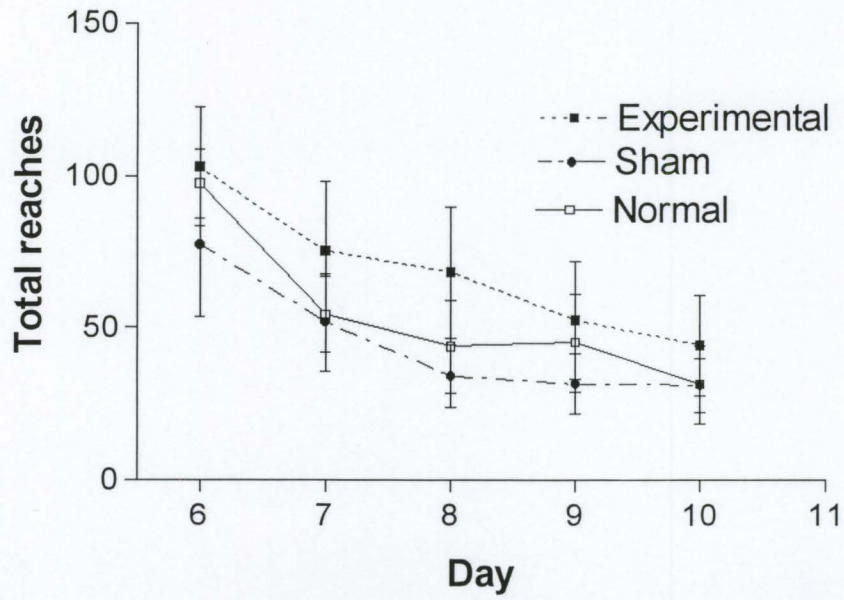
Figure 7:   The successful reaches divided by the total attempts made were used to

calculate the percent correct reaches made with the trained paw for

animals in the free-choice group. Graph **A** shows the percent correct

reaches made during the first block of training while graph **B** shows the

percent correct reaches made during the second block.

A

B

Figure 8:   The successful reaches divided by the total attempts made were used to calculate the percent correct reaches made with the untrained paw. Graph **A** are the percent correct reaches made during the first week of training while graph **B** records data collected during the second week of training. The animals were all in the free-choice group.

A

B

<u>Forced-choice reaching group</u>

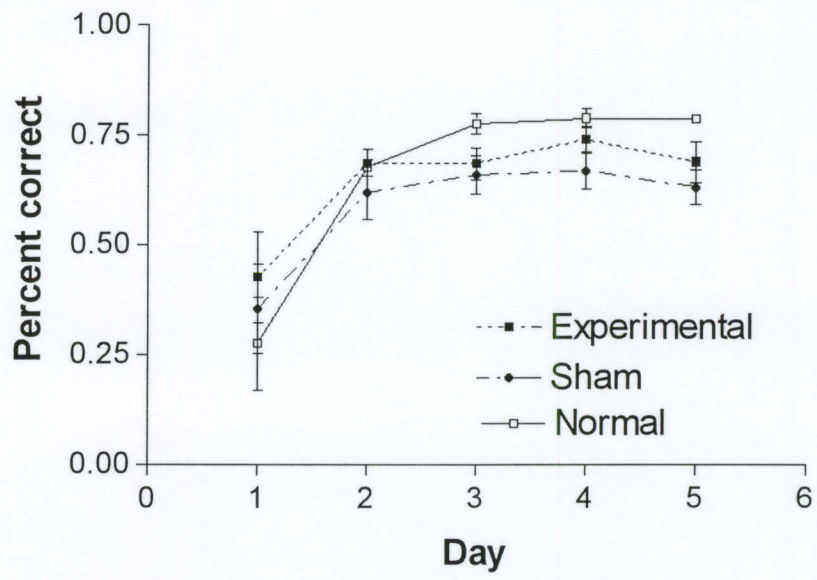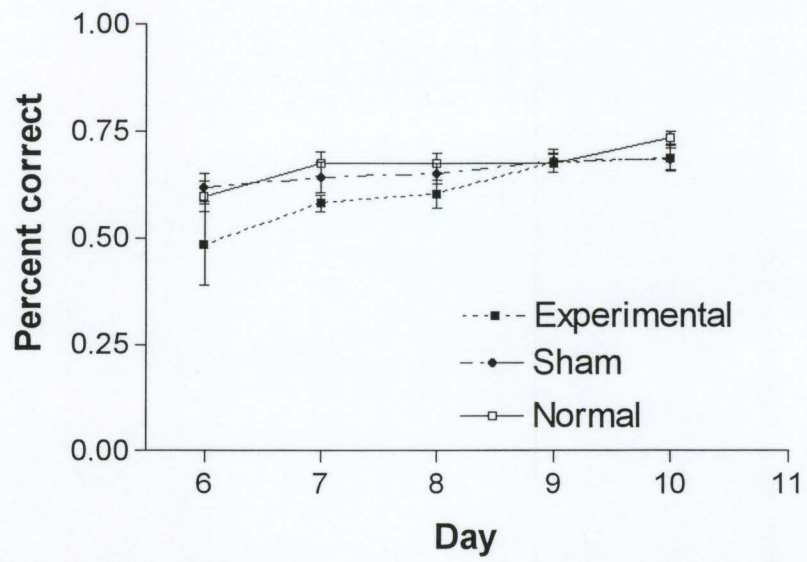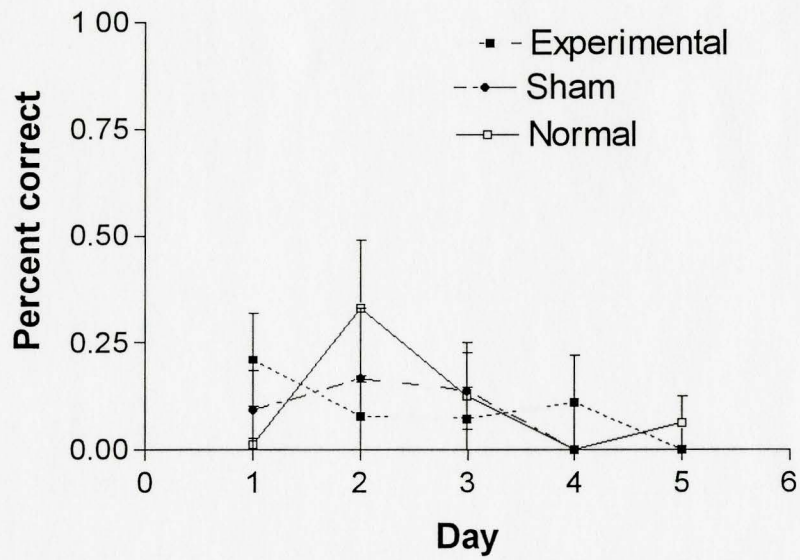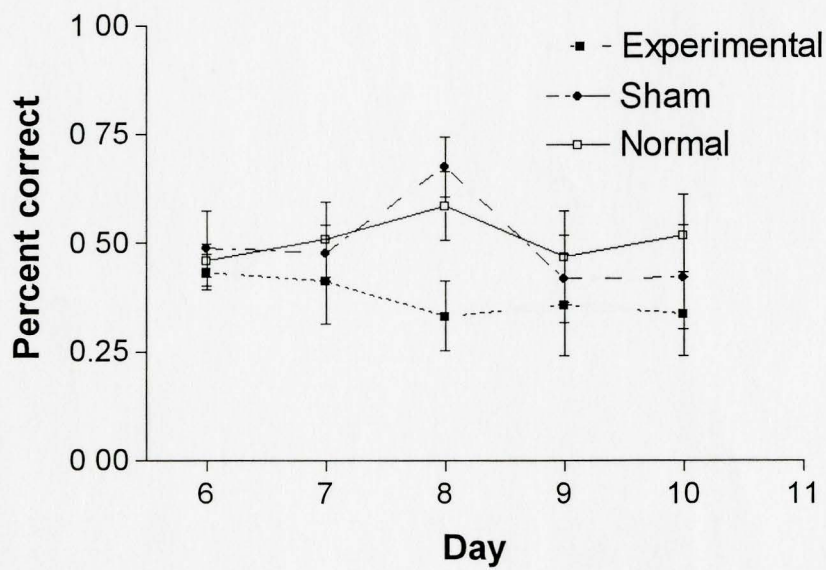This group consisted of 7 normal, unoperated animals. All data were analysed using one-way repeated measures ANOVA.

Analysis of the total number of reaching attempts using the trained paw in both weeks of training showed that there was a significant increase in reaching attempts across days ($F(4,24) = 30.0$, p< .001, and $F(4,24) = 20.2$, p<.001) (see Figure 9). For the untrained paw, there was a significant decrease in attempts across days ($F(4,24) = 5.56$; p<.01, and $F(4,24) = 4.32$, p<.01) (see Figure 10).

Using the percent correct reaches, the animals' performances on the reaching task were assessed. For the trained paw, in week one, there was a significant improvement in the animals' performances across days ($F(4,24) = 7.45$, p < .001). In the second week, performance did not change significantly across days ($F(4,24) = 1.97$, p =.13) (see Figure 11). For the untrained paw there was a significant decrease in use across days for week one but for week two of training, although use may have decreased, there was no significant change in reaching ability; ($F(4,24) = 4.05$, p =.01 and $F(4,24) =.63$, p =.64 respectively) (see Figure 12).

Figure 9:   The number of successful reaches and missed attempts were added to give

the total reaches. The data used to plot the graphs were collected from

animals in the forced-choice group. Graph **A** show the performance of the

animals using their trained paw in the first week of the experiment. Graph

B shows data collected for the trained paw in the second week of training.

A
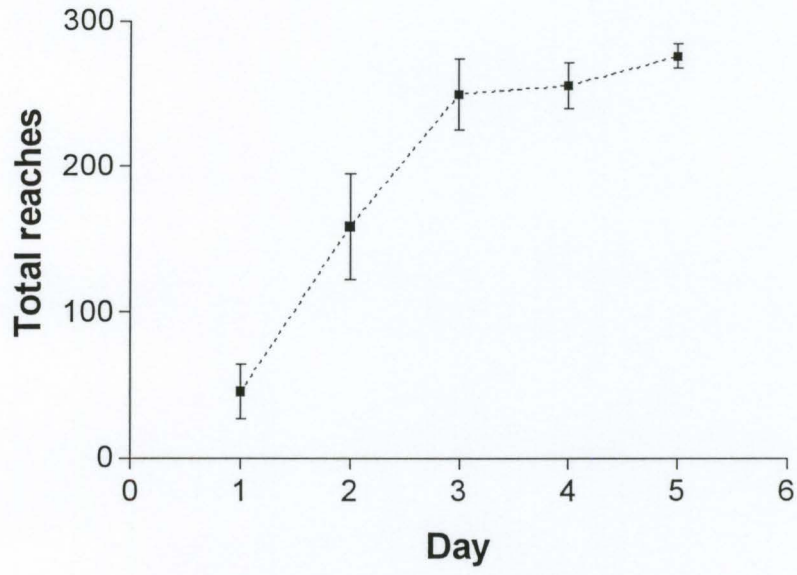
B
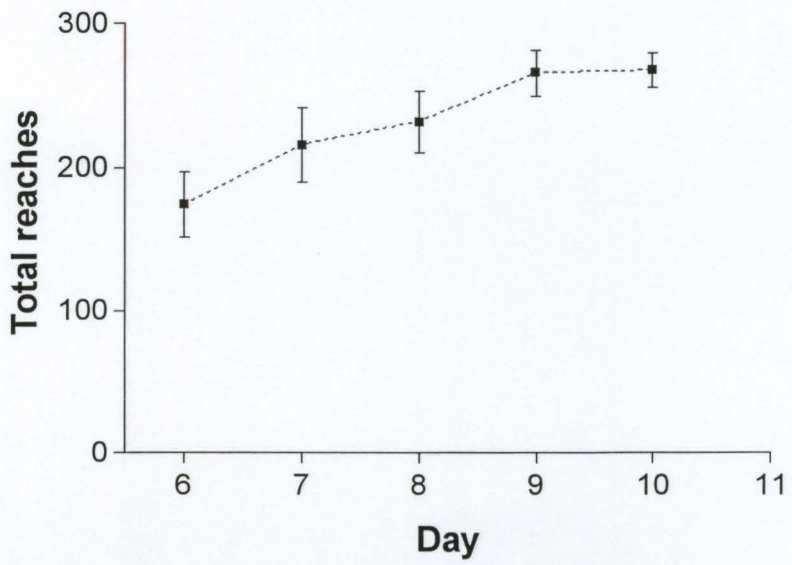
Figure 10: The number of successful reaches and missed attempts were added to give the total reaches. Data used were collected for the untrained paw of the animals in the forced-choice group. Graph **A** shows the attempts made in the first week of the task, while graph **B** shows attempts made during the second week.

A
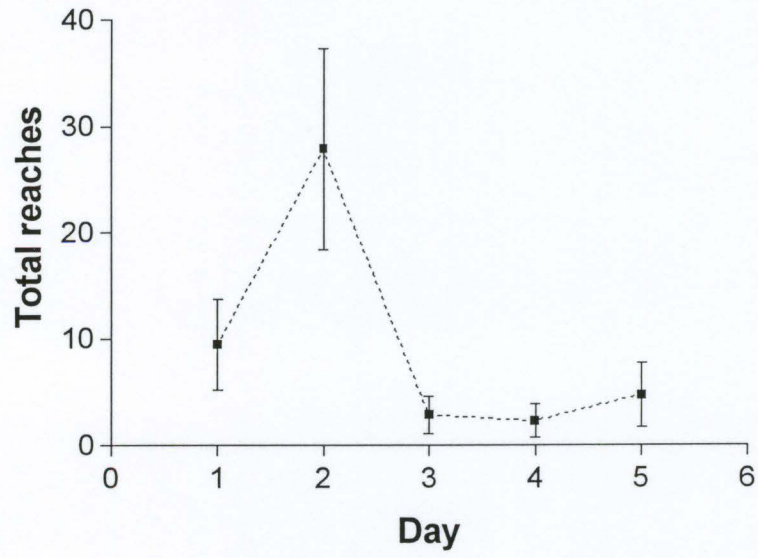
B
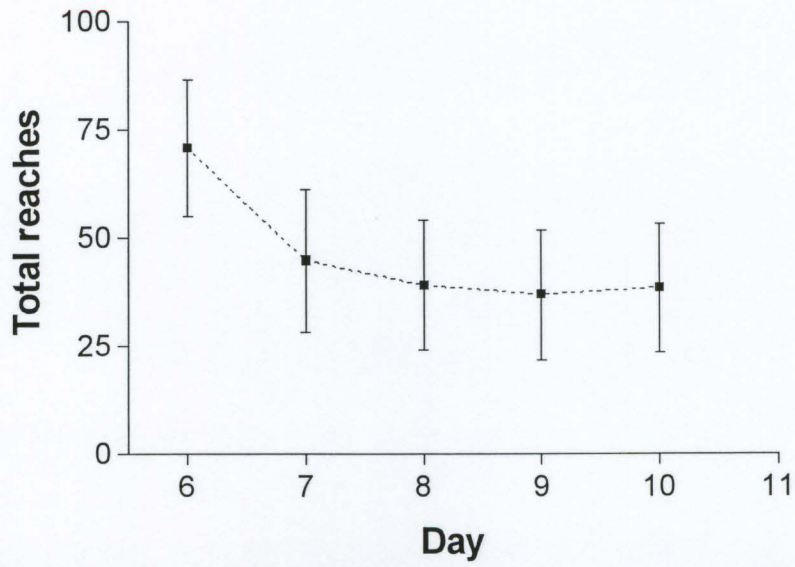
Figure 11:   The successful reaches divided by the total attempts made were used to

calculate the percent correct reaches for animals in the forced-choice

group. Graph **A** shows performance with the trained paw during the first

block of training. Graph **B** is a plot of performance with the trained paw

during the second block.

A
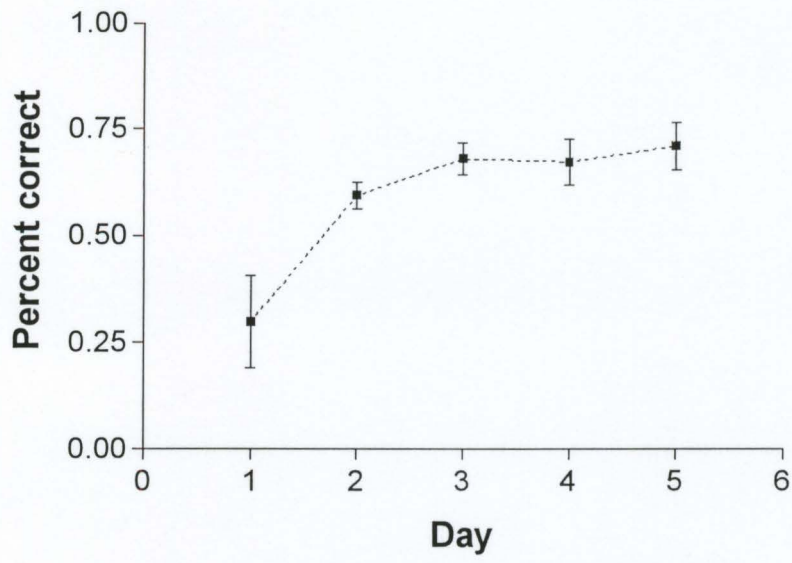
B
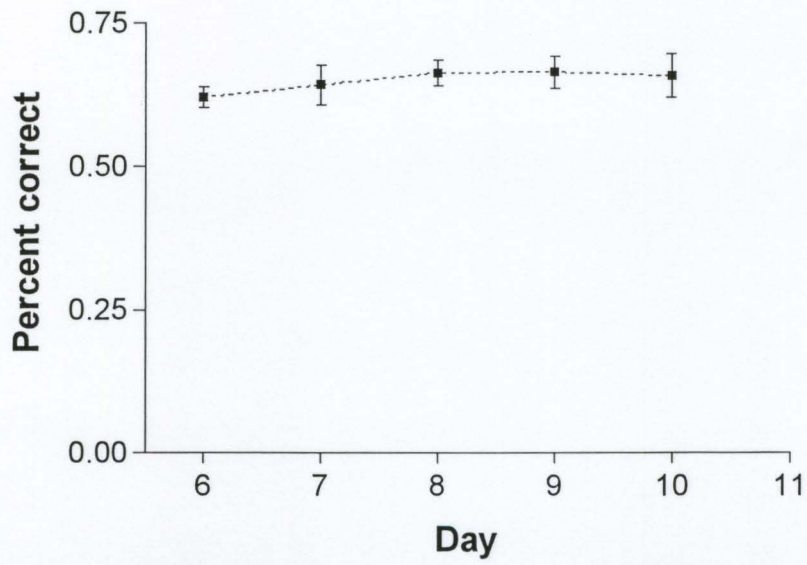
Figure 12: The percent correct reaches for the untrained paw were plotted for

performance in the first (graph **A**) and second (graph **B**) week of training.

The data used were collected from animals in the forced-choice group.
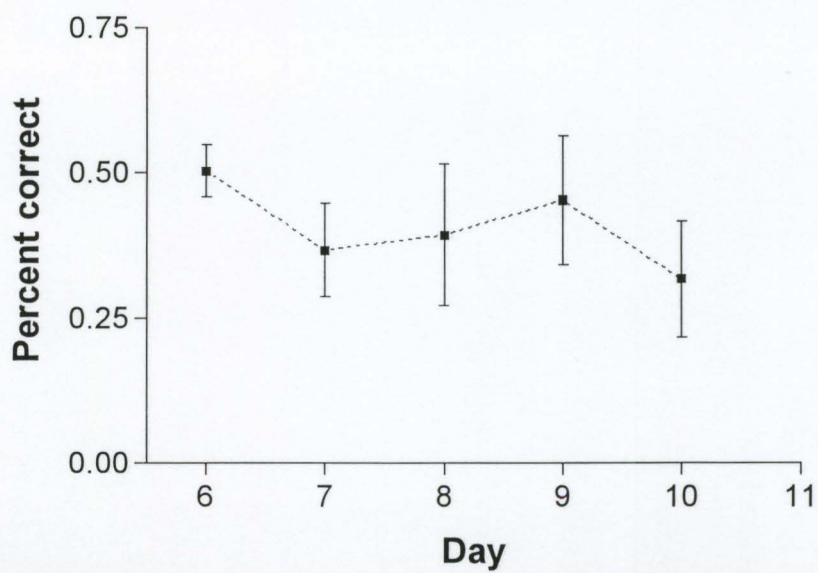
Comparison of the free and forced-choice groups

To see whether the acquisition of the task depended on which paw was used in the first week of training, the performance of the free and forced-choice groups of animals were compared. For the forced-choice reaching group, only normal animals were trained, so only normal animals were used for comparison with the free-choice reaching group. A plot of the total reaching attempts made by animals in both groups showed that they were equivalent (see Figure 13). There was a significant improvement in use of the trained paw across days in both training blocks ($F(4,52) = 83.9$, $p<.001$ and $F(4,52) = 46.6$, $p<.001$). There was no significant effect of group and no interaction effect ($F(1,13) = 2.56$, $p =.13$, and $F(1,13) = .41$, $p =.53$); and ($F(4,52) = .86$; $p =.49$, and $F(4,52) = 1.99$, $p =.11$ respectively). For the first training block, the untrained paw showed significant group, day and interaction effects for total reaches ($F(1,13) = 10.4$, $p<.01$; $F(4,52) = 7.21$, $p<.001$ and $F(4,52) = 5.21$, $p<.01$ respectively). For the second block, there was only a significant effect of day ($F(4,52) = 15.5$, $p<.001$) (see Figure 14).
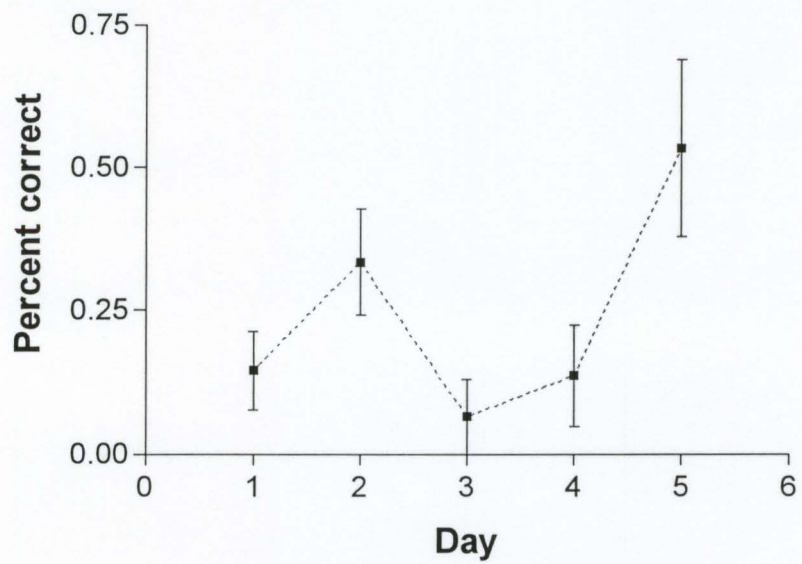
The animals in both the free and forced-choice reaching groups were equally adept at grasping and eating the food. Analysis of the trained paw revealed that in both weeks of training, the animals improved across days ($F(4,52) = 23.7$, $p <.001$; $F(4,52) = 8.85$, $p <.001$), but there were no significant effects of group placement ($F(1,13) = 3.66$, $p =.08$; $F(1,13) = .33$, $p = .57$) and no interaction effects ($F(4,52) = .46$, $p =.76$; $F(4,52) = 4.42$, $p= .24$) (see Figure 15). For the untrained paw, there was a significant decrease in use across days in the first, but not the second week of training; ($F(4,52) = 3.81$, $p<.01$;

$F(4,52) = 1.06$, p =.86). There were however no significant effects of group placement

and no interaction effects ($F(1,13) = 3.89$, p =.07; $F(1,13) = 1.42$, p = .25), and ($F(4,52) =$

2.39, p =.06; $F(4,52) = 1.06$, p = .38) (see Figure 16)

Figure 13:　A comparison of the total reaching attempts made by normal animals in the free and forced-choice groups. The data were collected for performance with the trained paw during the first (graph **A**) and second (graph **B**) blocks of training.

A

B
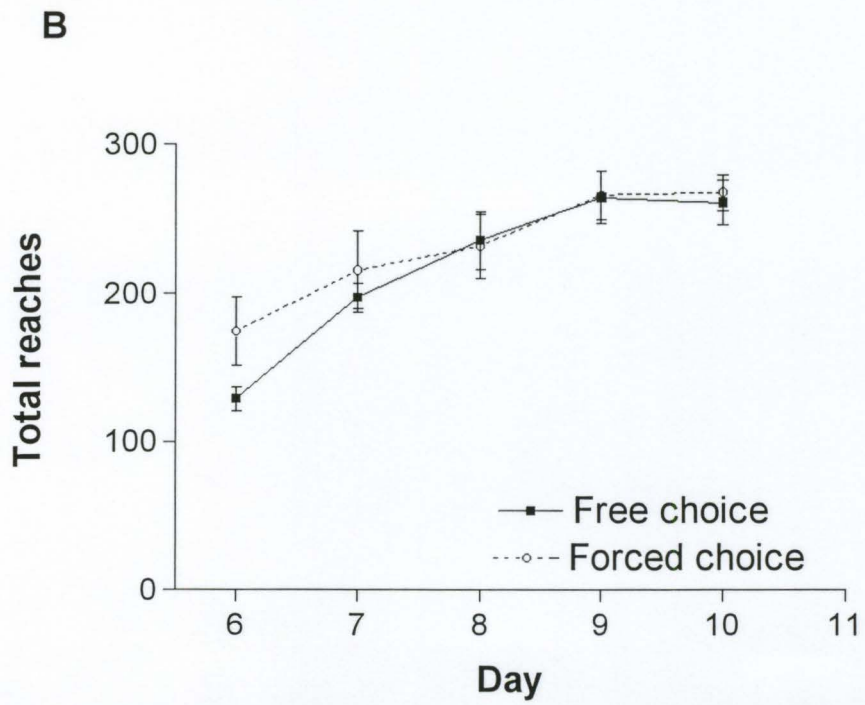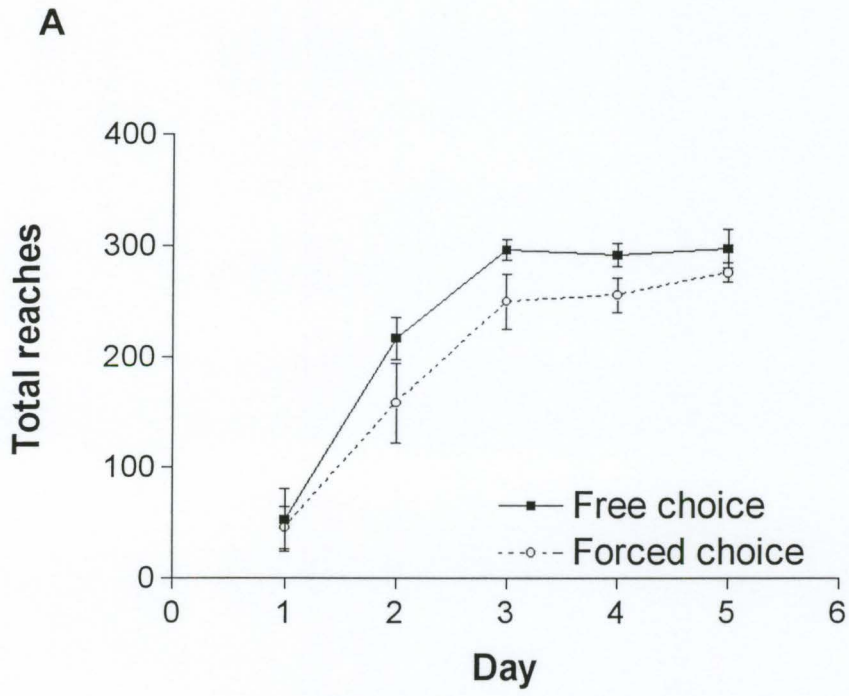
Figure 14:   The total reaching attempts made by the animals in the free and

forced-choice groups. The plot is of data collected for the untrained paw.

Graph **A** shows performance in block 1 while graph **B** shows performance

in block 2 of training.

A
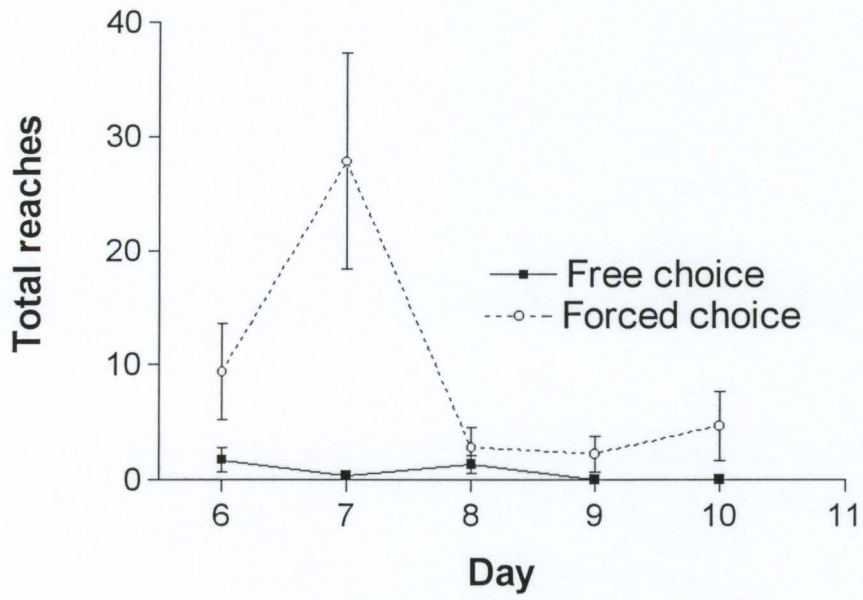
B
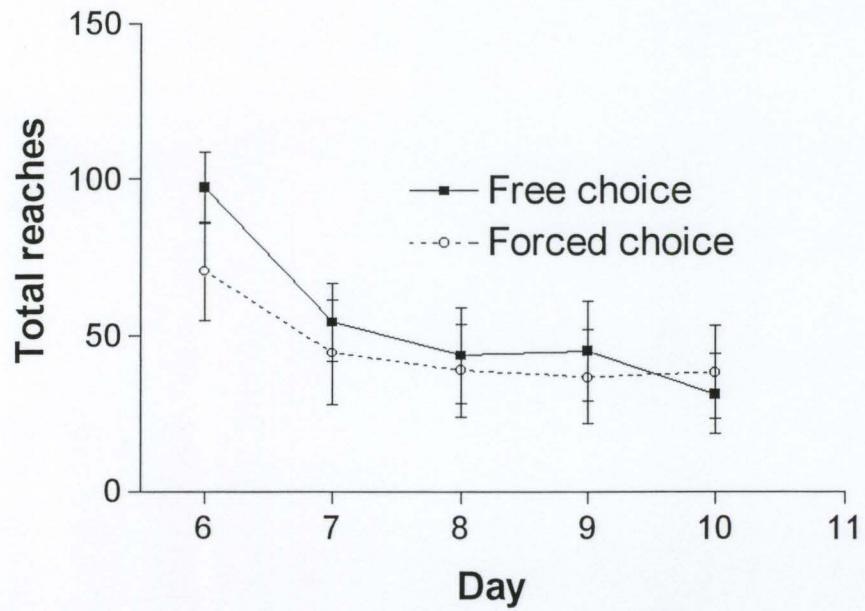
Figure 15:   Using the percent correct reaches, the performance of the normal control

animals in the free and forced-choice reaching groups was compared.

Graph **A** is a plot of reaches with the trained paw during the first block of

the experiment. Graph **B** is a comparison of performance with the trained

paw during the second block.

A
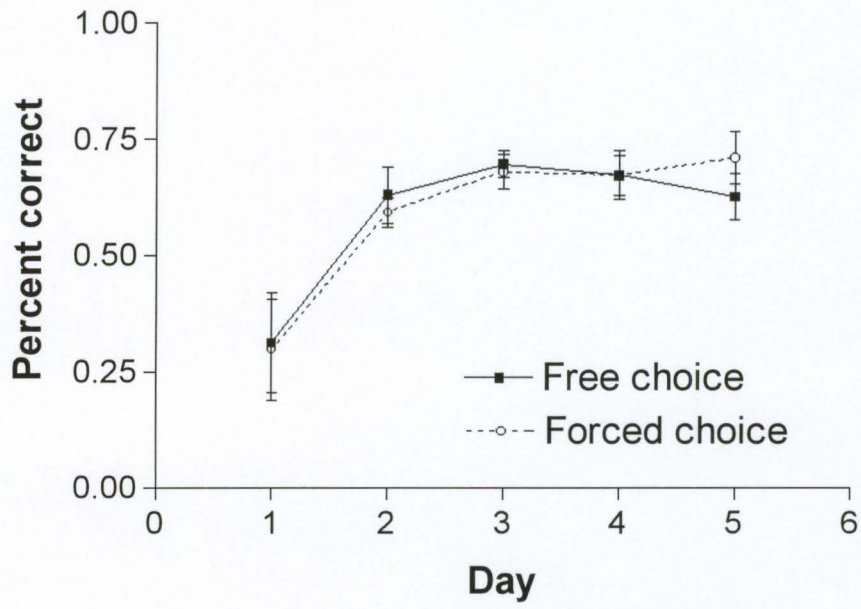
B
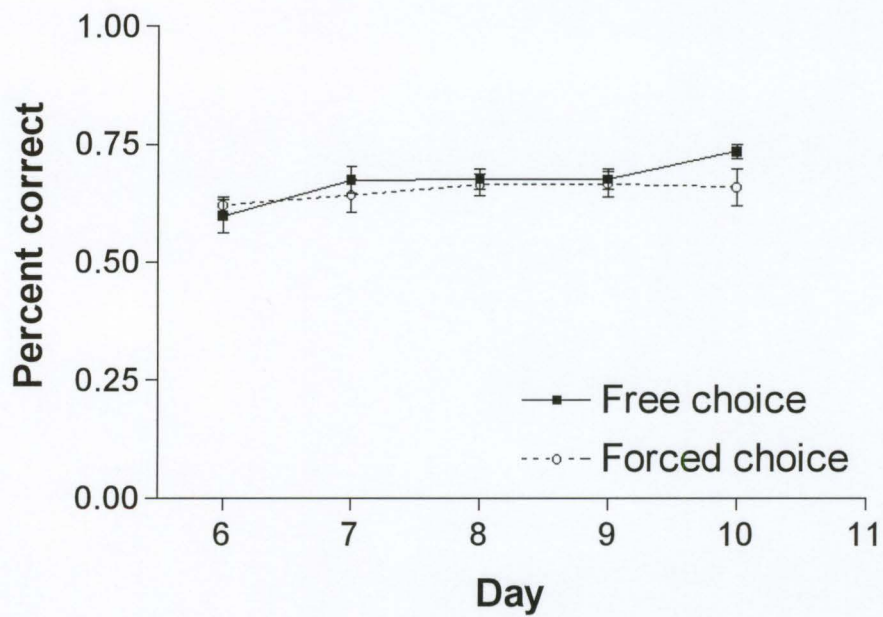
Figure 16:   The percent correct reaches with the untrained paw for the free and

forced-choice reaching groups. Graph **A** shows performance in the first

block while graph **B** shows performance during the second block of

training.

A
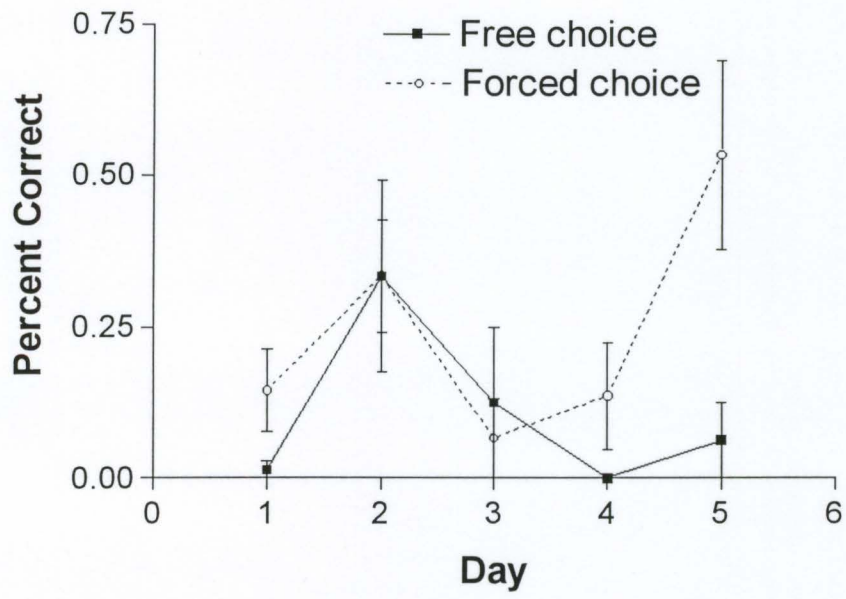
B
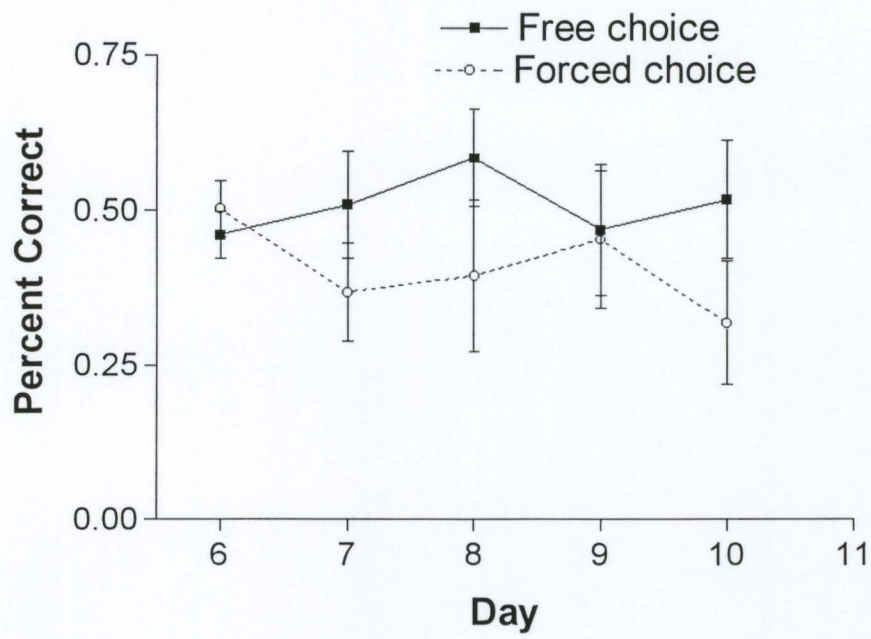
DISCUSSION

The results suggest that the corpus callosum may not be required for bilateral postural adjustments during the skilled reaching task, because there were no apparent postural differences between the groups. Although a new posture may be required and some learning involved, the callosum is not critical. Intact animals may use it for transferring information about balance, while transected animals may rely on a non-callosal strategy. In any case, casual observation of the animals suggests that balance in the experimental animals was unaffected by the callosal transection This conclusion is supported by other studies on split-brain subjects. In both human and non-human animals, no deficits in balance have been reported, even though the subjects may be impaired in other tasks involving hemispheric communication (Intriligator et. al, 2000; Mohr et. al, 1994). One reason for lack of impairment in balance may be that the primary centre for coordination of motor control is the cerebellum. Several studies have shown that animals with lesions to the cerebellum perform poorly on tasks that require motor coordination (Joyal et al., 1996; Le Marec et al., 1997). The animals in the present study are therefore capable of maintaining their balance because the cerebellum is intact and although inter-hemispheric coordination may be affected, the effect is not large enough to cause a noticeable difference in the arm-reaching task.

The results also suggest that the callosum may not be involved in the transfer of information about the skilled reaching task from the trained to the untrained hemisphere. This could be a Type 2 interpretation error, however, as a plot of these data shows that the intact animals may have had a slight advantage over the experimental animals in the first few days of the task (see Figure 7). The lack of statistical significance may be due to the relatively small sample size.

As mentioned above, several studies have shown that an intact callosum facilitates the transfer of information about a skilled task from one hemisphere to another (Hunter et al., 1975; Thut et al., 1997). It was therefore expected that the normal animals would perform significantly better than the experimental animals when training shifted to the untrained paw. The experimental group did drop from approximately 70% accuracy on the final day of block one to about 50% on the first day of block two, while the normal group only dropped from 79% to 70%. Even if this difference in performance loss is real, it may have been caused by factors other than the callosotomy. For example, the surgery may have caused additional damage to the brain that compromised performance. This explanation seems unlikely however, because the first block performance was comparable between the two groups. Most animals in all three groups of animals were reaching approximately 200 times by the second day of training and all were reaching approximately 300 times by the fifth day of training (see Figures 5A and 9A). These data showed that the motor performance was equivalent. Thus, any disadvantage suffered by the experimental group was not due to a nonspecific performance deficit due, for

example, to motor cortex damage. It could also be argued that experimental animals performed poorly in block two because they were more reluctant to switch from one paw to the other. There may have been a problem with inhibition of well-trained responses. However, all animals in the second training block showed comparable numbers of attempts to use their previously trained paw (see Figures 6B and 10B). The experimental group did not perform significantly differently from the other groups.

The purpose of the animals in the forced-choice group was to see whether there was an effect of paw preference that may have been a reflection of brain lateralisation. Unfortunately, because of lack of time, only a normal control group of animals could be trained, and these were compared with the normal animals that had been allowed to use their preferred paw in the first training block. If there was a strong laterality effect, then the free-choice group would have performed much more poorly during block two than the forced-choice group. However, both groups performed comparably during block two in terms of number of reaches (see Figure 13) or performance level (see Figure 15). Also, the animals gradually stopped using their untrained paw in the second week of training at an equivalent rate. The graphs showed that the animals were equally successful at performing the task and seemed to show that there was no effect of hemispheric dominance. These data agree with research carried out by Castro-Alamancos (1993) and Whishaw (1992). The study by Whishaw was carried out using Long-Evans hooded rats, the same strain used in the present study. Whishaw concluded that rats may prefer to use one paw over another, but this preference is not a reflection of dominance of one

hemisphere over another in reaching behaviour. Limb preference in the reaching task is only expressed when the animal begins reaching. The animal may, if tested on another task, use its other paw preferentially. The use of one paw over another is advantageous to the rats because it allows them to perform better in the reaching task. Whishaw found that animals that were ambidextrous performed more poorly than animals that showed a degree of lateralisation. To this end, animals that showed ambidexterity (animals that were still reaching with both paws on the third day of training) were excluded from the present study. The lack of evidence for hemispheric dominance in the skilled reaching task indicates that dominance is not a likely cause of any benefit or disadvantage that the animals gain from switching from one paw to the other. The possibility that the experimental animals were adversely affected in the skilled reaching task was discussed above. If this effect is real, it may reflect a deficit in the ability of the trained hemisphere to "teach" the untrained hemisphere how to perform the task. Work is currently underway to increase the number of animals in each of the groups in order to obtain a more conclusive result.

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