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Suppression to visual, auditory and gustatory stimuli habituates normally in rats with excitotoxic lesions of the perirhinal cortex

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Abstract

In 3 habituation experiments, rats with excitotoxic lesions of the perirhinal cortex were found to be indistinguishable from control rats. Two of the habituation experiments examined the habituation of suppression of responding on an appetitive, instrumental baseline. One of those experiments used stimuli selected from the visual modality (lights), the other used auditory stimuli. The third experiment examined habituation of suppression of novel-flavored water consumption. In contrast to the null results on the habituation experiments, the perirhinal lesions disrupted transfer performance on a configural, visual discrimination, indicating the behavioral effectiveness of the lesions. Implications for comparator theories of habituation are considered and we conclude that others' demonstrations of the sensitivity of object recognition to perirhinal cortex damage is not the result of standard habituation.

Keywords

perirhinal cortex; familiarity; novelty; habituation; recognition memory

Animals' initial responses to an unconditioned stimulus tend to decline with repeated presentation (see, e.g., Harris, 1943, Mackintosh, 1987) - that is, such responses *habituate*. This adaptive process is remarkably universal, being demonstrated in many response systems (e.g., Jordan, Strasser & McHale, 2000) and across many animal species (e.g., Jennings, 1906; Pinsker, Kuppermann Castellucci & Kandel, 1970; Tomsic, Pedreira, Romano, Hermitte & Maldonado, 1998; Wood, 1973). It is conceivable also that such an elementary process would contribute broadly to more complex psychological and adaptive processes. In particular, it is possible to interpret certain forms of object recognition in terms of simple habituation (e.g., Ennaceur, Neave & Aggleton, 1996).

Although, simpler accounts are available (and are probably essential in understanding invertebrate habituation), according to a general class of *comparator* theories (e.g., Konorski, 1967, Sokolov, 1963; Stein, 1966; Wagner, 1979), because a new stimulus is not already represented in memory its presentation will elicit the unconditioned response. But, as a consequence of its repeated presentation, that stimulus will come to be represented by the organism. It is the matching of incoming stimulus patterns with internal representations that is then assumed to suppress the unconditioned response. Sokolov (1963; see also, e.g., Konorski, 1967, Vinogradova, 2001) suggested that the familiar stimulus is represented as a cortical model, the matching mechanism being governed by the reticular system. The particular cortical region was not specified in these models, but one candidate is the perirhinal cortex.

The perirhinal cortex comprises areas 35 and 36, and in the rat is located in the rostral and mid portions of the rhinal sulcus (see, e.g., Burwell, 2001). The perirhinal cortex receives input from multiple sensory modalities (Burwell & Amaral, 1998), which would place it well for involvement for the development or storage of a cortical stimulus model during habituation. The perirhinal cortex is involved in recognition memory, as measured by electrophysiological studies and by behavioral tests of object recognition (see, e.g., Brown & Aggleton, 2001). Lesions of the perirhinal cortex have repeatedly been found to reduce recognition memory performance (see, e.g., Albasser, Davies, Futter & Aggleton, 2009; Liu & Bilkey, 2001; Mumby & Pinel, 1994; Mumby, Piterkin, Lecluse & Lehmann, 2007; Warburton, Kodor, Cho, Massey, Duguid & Barker, et al., 2003; Winters, Forwood, Cowell, Saksida & Bussey, 2004). Because recognition may be conceived as a process requiring comparison of a currently presented stimulus with a stored representation to judge prior occurrence (cf., Mandler, 1980), it is analogous to the comparator theories' analysis of habituation. As a consequence, the role of the perirhinal cortex in recognition might be taken to indicate that it also plays a role in habituation (cf., Fernández & Tendolkar, 2006). On that basis, we intended to investigate the effects of excitotoxic perirhinal lesions on the habituation of rats' responses. If response habituation is, in part, governed by a cortical model (Sokolov, 1963) and if that model is located in the perirhinal cortex, we would expect to see attenuation in the rate of habituation. Such a finding would encourage the notion that normal object recognition could be the result of a relatively simple process of habituation; but a failure to find any effect of perirhinal cortex lesions on habituation would suggest that object recognition relied on some distinct process.

We have recently reported data showing perirhinal lesions to have no apparent affect on habituation (Albasser, Davies, Futter & Aggleton, 2009). However, this observation was based on a single null result and used a rather specialised habituation procedure. To more fully assess the role of the perirhinal cortex in habituation, we performed three habituation experiments, each employing stimuli from a different modality: visual, auditory and gustatory, all of which are well represented in the perirhinal cortex (see, e.g., Burwell, 2001; Burwell & Amaral, 1998). Our employment of stimuli from multiple modalities was intended to provide a relatively robust examination of any role of the perirhinal cortex in habituation.

Experiment 1

Before habituation training, two groups of rats were given either excitotoxic injections, designed to produce bilateral perirhinal damage (group PeRh), or received a sham surgery (group Sham). The rats were then compared on the habituation of their orienting response to a repeatedly presented light, a behavior that has previously been well characterised (e.g., Hall & Channell, 1985; Jordan et al., 2000). Our procedure was modified in that we measured the orienting response indirectly by assessing the extent to which the light's presentation modified appetitive activity. Here, rats earned food pellets on a free-operant schedule and presentation of the light was expected to produce an orienting response that would suppress instrumental behaviour (see, e.g., Hall and Honey, 1989; Gustafson & Koenig, 1979; Jordan, et al., 2000). Habituation of this response would take the form of a reduction in the suppression of instrumental behaviour with repeated light presentation.

Subjects

Sixteen male, Dark Agouti rats (B & K Universal Ltd, UK) served as subjects and were randomly assigned to either group Sham or group PeRh ($n_s = 8$; see, *Surgery* below). Except during experimentation (see *Procedure* below), rats were held in an air-conditioned vivarium that was illuminated by fluorescent strip lights between 0700 - 1900. Temperatures were maintained between 20 and 23°C. Rats were pair-housed in acrylic cages. Cages contained fresh woodchip bedding and tap water was always available. Rats received free access to food (Harlan Teklad, Bicester, United Kingdom) in the cages until one week before the experiment began (see *Procedure* below). At that time, rats' weights were recorded (group-Sham mean: 255.0 g; range: 230 – 285 g; group-PeRh mean: 252.5 g; range: 235 – 275 g) and food access was thenceforth restricted. Measured amounts of food were given once daily to reduce gradually rats' weights to between 80% and 90% of their baseline weight.

Surgery

Rats were anesthetized using an intraperitoneal injection of pentobarbitone sodium (Sagatal, Rhone Merieux) at a dose of 60 mg/kg body weight. Next, their scalps were shaved and they were placed in a stereotaxic headholder (Kopf Instruments, Tujunga, CA). The incisor bar was set to +5.0 of the horizontal plane before the scalp then the temporal muscles were retracted, exposing the skull. Next, an area of the skull above each hemisphere's parietal cortex was removed, approximately 4–7mm posterior to bregma. Microinjections were made with a 1μL-syringe (Hamilton, Switzerland). Anterior-posterior (AP) and medial-lateral (ML) placements were: AP + 4.2, ML $\pm$ 5.6; AP + 2.7, ML $\pm$ 6.1; AP + 1.3, ML $\pm$ 6.2, relative to ear-bar zero). The depth, from bregma, at each site was 9.2 mm (most rostral), 9.4 mm, and 9.0 mm (most caudal). For rats in group PeRh, 0.26-μL bilateral injections of 0.09M N-methyl-D-aspartic acid (NMDA, Sigma Chemicals, UK) were made at each AP level, with the needle left in situ for four min. For rats in group Sham, the syringe needle was placed in the same location but no injection was delivered. Following surgery, the scalp was sutured, and an antibiotic powder (Acramide; Dales Pharmaceuticals, Skipton, UK) was topically applied. Rats also received subcutaneous injections of 5 mL glucose saline.

After completion of Experiment 4, the rats in both groups (PeRh and Sham) were anaesthetised with sodium pentobarbital (1 mg/kg) and intra-cardially perfused with 0.1-M saline, followed by a 5% formal saline solution. The rats' brains were then removed and post-fixed in a 5% formal saline solution. Before sectioning, brains were steeped overnight in 25% sucrose solution. Coronal sections were cut at 40 μ m using a freezing microtome. Every third section was kept and mounted onto gelatine-coated slides, which were then stained with cresyl violet. Sections were viewed microscopically to determine the extent and location of excitotoxic damage by reference to a brain atlas (Paxinos & Watson, 2005)

Apparatus

Eight identically specified Skinner boxes supplied by Campden Instruments Ltd. (Loughborough, UK) were used. The boxes were individually housed in light- and sound-attenuated chambers that were equipped with an exhaust fan serving to ventilate the boxes and to provide a background sound pressure level of 67 dB (A). Each box had three aluminum walls and an aluminum ceiling; a transparent plastic door served as the fourth wall. The floor was made from stainless steel rods, 5 mm in diameter and spaced 15 mm apart. The internal dimensions of a box were: 24.5 cm wide; 23.0 cm deep; 21.0 cm high. The boxes were equipped with a concave, recessed tray to which 45-mg food pellets could be delivered (Formula P, P.J. Noyes, Lancaster, NH). A sprung, transparent plastic flap (6 cm high, 5 cm wide) covered this tray. Pushing the flap actuated a microswitch, which was recorded as a response. A 2.8-W, incandescent lamp was located behind the flap and 8 cm above the tray. Each box also had four, 2.8-W incandescent lamps covered by 1.5 cm diameter plastic disks. One lamp was located centrally in the ceiling and three were located on the wall that housed the food tray. Only the tray and ceiling lamps were used (see *Procedure*, below). A RISC PC microcomputer (Acorn Computers Ltd., Cambridge, England), programmed in Arachnid (Paul Fray Ltd, Cambridge, England), was used for the control of the experimental events and the recording of responses. A heavy-duty relay, located on the ceiling, could be operated at 10 Hz to produce an 80-dB (A) train of clicks and a loudspeaker, (also located on the ceiling), could be used to present a 2-kHz and < 85-dB (A) pure tone. Stimulus sound-pressure level measurements were taken against the background level of 67 dB (A). Auditory stimuli were used in Experiment 2 (see below) but not in Experiment 1.

Procedure

Rats were first trained to retrieve pellets from the food tray. In each of two sessions, pellets were delivered to the tray on a 1-min, fixed-interval schedule. In the first session, the flaps that cover the food tray were held open with electrician's tape; in the second session, as in the rest of the experiment, the flap was not held open. Flap pushing was established as an instrumental response. A 60-s, variable interval (VI60; range: 45 – 75 s) schedule of reinforcement was established and maintained throughout the remainder of the experiment.

The effect of presentation of a light stimulus on appetitive responding (orienting response) was assessed over the next four, 45-minute sessions, which were run on separate, successive days. During assessment of the orienting response, VI60 reinforcement remained in operation for flap pushing. We chose to use two lights, each differing in several respects:

The tray light produced a relatively localized light, clearly illuminating the rectangular tray door but producing little reflection on the Skinner box walls; the ceiling light produced a diffuse light, whose reflection was apparent on the four walls. Furthermore, the ceiling light was presented pulsed (1 Hz on/off); the tray light, without such interruption. For half of the rats in group Sham and group PeRh, the tray light was used; for the remainder, the ceiling light was used. Both lights were 1-min duration and occurred twice in each session with a variable inter-trial interval (ITI; mean: 16 minutes; range: 8.5 – 22.5 minutes, measured from either the point of the program's actuation or the previous trial's termination to the onset of the trial). Responding was recorded during each trial in four, 15-s bins to allow fine-resolution analyses of changes in flap pushing. Stimulus presentation had no effect on the probability of obtaining food reinforcement.

Results & Discussion

Histology

The perirhinal cortex comprises areas 35 and 36 (cf., Burwell, 2001). All eight rats in group PeRh sustained substantial, bilateral damage to the perirhinal cortex. Fig. 1 depicts the extent of tissue loss in the case with the largest and the case with the smallest lesions. In four cases the tissue loss included almost the entire length of the region, and so extended rostrally as far as the agranular insular cortex (Paxinos & Watson, 2005). In four others there was limited, unilateral cell sparing in the most rostral portion of perirhinal cortex (Fig. 1). In every case the lesions extended to the caudal border with the postrhinal area. In some cases there was partial sparing of either the deep or superficial lamina within the perirhinal cortex at particular AP levels (Fig. 1). Even so, in the majority of sections the perirhinal cell loss involved all lamina. All rats also had at least some extra-perirhinal cortex damage, most notably in the lateral entorhinal cortex and the piriform cortex, which was variable and sometimes bilateral. The lesions often encroached dorsally into the ventral part of association area, TeA, which is immediately adjacent to the perirhinal cortex. In five cases there was very restricted cell loss in the caudal part of CA1, adjacent to the fundus of the rhinal sulcus. In one further case this restricted CA1 cell loss was bilateral. In one case the lesion extended into the lateral amygdala nucleus in one hemisphere. All PeRh surgical cases were retained for behavioral analyses.

Behavioral Findings

Appetitive training proceeded successfully and did not differ between groups. For example, mean response rates on the final session were: for group Sham, 11.6 responses per minute; and, for group PeRh, 12.5, $t < 1$. The extent of the effect of the lights on flap pushing was assessed using a normalized difference score: $(L - p)/(L + p)$, where L is the rate of responding during light's presentation; p is the rate during the 1-minute period immediately preceding that light presentation. The ratio will produce values between +1 and -1, with positive ratios corresponding to trials whose light rates exceed pre-light rates (i.e., *elevation*) and negative ratios corresponding to trials whose light rates fall short of pre-light rates (i.e., *suppression*), and with 0 corresponding to parity. Both lights (tray and ceiling) produced similar levels of orienting response. For example, a preliminary analysis of variance

(ANOVA; otherwise identical to that reported immediately below) failed to produce a main effect or any interactions, of the light-identity factor, all F s < 1.

Data collapsed over the light factor are summarized in the left panel of Fig. 2. It is apparent that the light tended to suppress flap pushing (i.e., all mean ratios are negative) and that the suppression was particularly pronounced on the first trial, recovering to some extent on subsequent trials. But the perirhinal lesions appeared to have no influence on the level or recovery time-course of that suppression. ANOVA with session (four levels), trial (two levels) and lesion factors confirmed these impressions: (a) the intercept indicated that, pooled over both of the groups and all eight trials, the ratios were less than 0, $F(1, 14) = 70.97$; $p < .001$; (b) the main effect of trial was significant, $F(1, 14) = 15.04$; $p < .002$; but that (c) no other main effect nor interaction was significant, smallest $p > .249$. Thus, the ANOVA indicated that the within-session habituation (i.e., from the first to the second trial, collapsed over all four sessions) carried the habituation, rather than the any between-session habituation, which would have yielded either a main effect of session or a Session $\times$ Trial interaction. Despite that, inspection of the figure indicates that the greatest trial 1-trial 2 difference was during session 1. One noticeable feature of the orienting response to light stimuli is the speed with which it declines (Hall & Channell, 1985; Jordan et al., 2000). That fact raises the concern that a genuine, lesion-induced deficit in habituation was obtained but masked by a relatively crude measure of the orienting response. Although we have no reason to doubt the general sensitivity of trial-by-trial data – which successfully detected habituation here – we were able to examine within-trial habituation whose greater resolution may permit detection of a group difference of the course of habituation. To test this suggestion we examined the first light trial's data as four, 15-s bins. Bin responding was converted to a response-per-minute rate to match that of the 60-s pre-light period. This permitted meaningful conversion to normalized difference ratios, which are summarized in the right panel of Fig. 2. On this single trial also, there was no apparent influence of perirhinal lesions. ANOVA with bin and lesion factors revealed no main effects nor interaction, smallest $p > .175$; but the intercept was significant, $F(1, 14) = 71.72$; $p < .001$, indicating significant suppression of flap pushing during the light.

Our observation that the orienting response to the light declined with repeat presentation matches standard descriptions of habituation (e.g., Harris, 1943; Thompson & Spencer, 1966). However, we failed to find that lesions of the perirhinal cortex affected the course of that habituation – a finding acting to disconfirm the suggestion that the perirhinal cortex supports the cortical model, suggested by certain comparator theories of habituation (e.g., Sokolov, 1963). The next experiment sought to examine the same question with the same rats, but using stimuli from a different modality.

Experiment 2

The current experiment examined the impact of perirhinal cortex damage on habituation to an auditory stimulus. Burwell and Amaral (1998) reported that the proportion of auditory afferents to the perirhinal cortex was greater than that for visual afferents, and so one might expect an auditory stimulus to provide a relatively sensitive test of perirhinal involvement in habituation. To promote comparison with the results of Experiment 1, the current

experiment employed its response measure (suppression of instrumental responding) and same general procedure, differing only in its use of auditory, rather than visual stimuli.

Method

Subjects and Apparatus

The rats and apparatus were those of Experiment 1. Rats' maintenance was the same as in Experiment 1. None of the lamps described in Experiment 1 were used to illuminate the Skinner boxes at any point during Experiment 2.

Procedure

Rats' flap pushing continued to be reinforced as it had been in the previous experiment. No light stimulus was used; rather, a 1-minute, 2-kHz tone or 10 Hz click stimulus was presented (see *Apparatus*, Experiment 1). For half of the rats in group Sham and half of the rats in group PeRh, only the tone was presented; for the remainder, only the click was presented. Trials were scheduled to occur separated by the variable 16-minute ITI described in Experiment 1. Rats received four such daily sessions.

Results & Discussion

Rats' baseline response rates remained stable throughout the experiment and at a similar level to that of Experiment 1. The introduction of auditory stimuli produced marked changes in response rate (see left panel of Fig. 3). A preliminary ANOVA (analogous to that described below) produced an interaction between auditory stimulus (i.e., clicker versus tone) and session, $F(3, 36) = 7.21$; $p < .001$; but, of most importance here, there were no interactions involving the group and stimulus factors, smallest $p > .131$. Unlike the habituation to visual stimuli seen in Experiment 1, the most prominent changes in unconditioned responding to the auditory stimuli of Experiment 2 occurred over the four sessions and so data were pooled over the two trials of each session for statistical analysis. Like the lights used in Experiment 1, the auditory stimuli produce suppression, but only early in testing. Later in testing, auditory trials produced *elevation*. Despite the response being biphasic – thereby extending the scope for detection of the involvement of the perirhinal cortex in unconditioned responding – there was no obvious difference in group Sham and group PeRh's unconditioned responding. ANOVA with session and lesion factors yielded neither a main effect of lesion, nor an interaction with session, smaller $p > .290$. The main effect of session was reliable, $F(3, 42) = 6.21$; $p < .002$. Polynomial contrast-analyses revealed responding over sessions was best characterized as quadratic: Linear and quadratic trends were reliable, smaller $F(1, 14) = 7.12$; $p < .018$; the cubic trend was not, $p > .546$. The intercept was not reliable, $p > .698$, reflecting the ratios' approximate symmetry around 0. However, one-sample t tests on session-1 and session-4 data revealed that both differed from 0, smaller $t(15) = 2.45$; $p < .026$, thereby demonstrating initial suppression, then, *elevation* of flap pushing.

Fine-resolution analysis of trial 1 is summarized in the right panel of Fig. 3 in which ratios were computed for four, 15-s bins. But here also, group Sham and group PeRh's performance was similar. ANOVA with lesion and bin revealed neither the main effects nor

the interaction, smallest $p > .207$, though the intercept was reliable, $F(1, 14) = 19.81$; $p < .001$, indicating that the auditory stimulus provoked suppression of responding relative to the immediately preceding one-minute period. The results of the current experiment, therefore, confirmed and extended those of Experiment 1. Once again, lesions of the perirhinal cortex did not affect the course or extent of habituation, but the finding that the auditory stimulus produced biphasic responses (i.e., both suppression and elevation) extended the range of scope for detection of a lesion effect.

Experiment 3

While both Experiments 1 and 2 failed to find evidence that perirhinal cortex lesions disrupt habituation, these demonstrations are potentially narrow in scope as both employed exteroceptive stimuli and both used the suppression of instrumental responding as the indicator measure. Furthermore, there is reason to believe that the perirhinal cortex may have a diminished role in the recognition of auditory stimuli (Fritz, Mishkin & Saunders, 2005; Kowalska, Kusèmierek, Kosmal & Mishkin, 2001; Wan, Warburton, Kusmiereck, Aggleton, Kowalska & Brown, 2001). The purpose of the current experiment was to extend the measure of habituation to a non-exteroceptive stimulus (gustation) and a different response system (non-instrumental drinking). Because, area 35 of the perirhinal cortex receives a higher proportion of inputs from gustatory regions (gustatory area and gustatory insular cortex) than from visual areas (Burwell & Amaral, 1998), this modality seems most appropriate with which to test the impact of perirhinal cortex lesions. In the current experiment, rats were offered water that was flavored with a gustatory stimulus that provokes suppressed consumption, which habituates with repeat presentation.

Method

Subjects & Apparatus

The subjects were those used in Experiments 1 and 2. After Experiment 2 ended, rats were given two-weeks' free access to both food and water in their home cages to promote recovery from weight loss. Rats remained in their home cages (see, *Subjects*, Experiment 1) throughout Experiment 3, where food was freely available but where fluid accessibility was restricted (see *Procedure*, below). De-ionized water was used to make solutions of 0.01-M HCl and 60.00- μ M quinine hydrochloride, which could be presented to rats in inverted 50-mL centrifuge tubes, fitted with ball bearing-tipped spouts. To the experimenter, the solutions tasted, respectively, weakly sour and weakly bitter. A balance was used to record rats' fluid consumption to the nearest 0.1 g.

Procedure

Before the experiment began, a schedule of water restriction was initiated: Home water bottles were removed overnight and, on each of the next 3 days, water was offered once per day for one hour (bottles on at 10:00). Following this, rats were given a free-access trial to flavored fluid once per day for four days, for one hour (bottles on at 10:00). For half of the rats in group Sham and half of the rats in group PeRh, HCl solution was offered; for the

remainder quinine hydrochloride was offered. Rats' consumption was recorded once after 15 minutes, then again after 45 minutes.

Results & Discussion

A preliminary ANOVA (analogous to that described below) produced no significant statistics involving the flavor factor (i.e., quinine versus HCl) and any other factor, smallest $p > .073$. The results of Experiment 3 are summarized in Fig. 4. Despite the second (45-minute) period of each trial being three times the duration of the first (15-minute) period, less than half the weight of fluid was consumed then – a feature of our data probably reflecting a reduction in rats' thirst during that first period. Also apparent, in both periods, is that consumption was relatively slight on the first trial, increasing on subsequent trials. We take this pattern of consumption to reflect the habituation of neophobia to the flavor. As in the previous experiments, habituation appeared to be uninfluenced by the perirhinal lesions; that is, the course of habituation was similar in groups Sham and PeRh. This description of the data was supported by ANOVA with group, period and trial factors, which revealed main effects of period, $F(1, 14) = 70.98$; $p < .001$, and session, $F(3, 42) = 11.40$; $p < .001$, but no main effect of lesion nor interactions, with smallest $p > .147$. Polynomial contrast analyses revealed that responding over the four daily trials was best characterized as quadratic: Linear and quadratic trends were reliable, smaller $F(1, 14) = 13.72$; $p < .003$; the cubic trend was not, $p > .752$.

In the current experiment we sought to examine a possible role for the perirhinal cortex in habituation of a neophobic response, but found no such support. Experiment 3, then, joins Experiments 1 and 2 in showing the course of habituation in rats with perirhinal lesions to be indistinguishable to that of rats with sham control surgery.

Experiment 4

The apparent failure of perirhinal lesions to affect the course of habituation in Experiments 1-3 can be seen as evidence of an important role for extra-perirhinal brain areas for this behavior. Such reasoning assumes the efficacy of the surgery, which was tested in Experiment 4 using a different type of learning problem.

Rats with perirhinal cortex lesions have been found to be deficient in certain discrimination problems. For example, Campolattaro and Freeman (2006) trained rats on a feature-positive discrimination, having the form A-/AX+, where A and X were brief auditory and visual stimuli, that occurred as a *simultaneous* compound on AX trials, and + was a periorbital-shock reinforcer. Before discrimination training began, Campolattaro and Freeman gave one group of rats perirhinal cortex lesions and that group's discrimination was attenuated relative to an appropriate control group. Campolattaro and Freeman's report included another lesion and control group that was also given a feature-positive discrimination – but with the X and A presented as a *serial* compound. Analytical experiments of feature-positive discrimination in rats have encouraged the view that processing of a compound stimulus is configural in nature when its elements are presented simultaneously, though not when presented serially (e.g., Holland & Lamarre, 1984). The implication, therefore, is that

Campolattaro and Freeman's perirhinal lesions produced a selective effect on a configural learning process.

The observation that rats with perirhinal cortex lesions are hindered in their solution of configural discriminations has been reported in a number of studies using a variety of procedures (e.g., Bucci, Saddoris, Burwell, 2002; Bussey & Saksida, 2002; Campolattaro & Freeman, 2006; Dusek & Eichenbaum, 1998; Eacott, Machin & Gaffan, 2001; though see, Bussey, Dias, Redhead, Pearce, Muir & Aggleton, 2000; Davies, Machin, Sanderson, Pearce & Aggleton 2007). We sought to demonstrate the efficacy of our perirhinal cortex lesions using a variation of a simultaneous visual discrimination (e.g., George, Ward-Robinson & Pearce, 2001), using apparatus depicted on the top left panel of Fig. 5. The same control and perirhinal cortex lesioned rats as those used in Experiments 1-3 were trained in a water tank to swim towards certain visual patterns (cf., Alvarado and Rudy, 1995), but the arrangement of elements within the transfer test required a configural solution, (cf., Norman & Eacott, 2004).

Rats were first trained on a simultaneous discrimination in which they were required to discriminate between a pair of compound stimuli composed of four black and white elements: a black rectangle (B); a white rectangle (W); black and white horizontal stripes (S); and, a white ellipse (E). Two compounds were used: B|S (B on the left of S); and W|E (W on the left of E; see the bottom left panel of Fig. 5. The two compounds were presented simultaneously on each trial (i.e., all four of the stimulus elements were presented) and rats learnt to use the location of these compounds to locate an escape platform, which was located immediately below one of the two compounds (e.g., B|S). Conversely the escape platform was never located below the alternative compound (e.g., W|E). On half of the trials the arrangement of the compounds was that depicted in the bottom left panel of Fig. 5 (i.e., B|S left of W|E) but on the remainder of the trials, W|E was left of B|S. The particular assignment of compound and escape platform was counterbalanced (see right panel of Fig. 5, *Training*). The lateral position of the compounds did not affect their reinforcement; for example, rats receiving reinforcement to B|S did so whether it appeared on the left or the right side. Following their mastery of the simultaneous discrimination, rats were given the crucial transfer test in which one of the original training compounds remained (e.g., B|S) but the other (here, W|E) was replaced with the mirror image of the remaining original compound (here, S|B, see right panel Fig. 5, *Test*). For all rats, the original compound indicated the presence (or absence) of the escape platform, precisely as it had during Training – and the mirror-image compound indicated the reverse arrangement. Notice that efficient transfer test performance may be most naturally thought of requiring a configural solution because all its elements (i.e., B, S, left and right) are equally often reinforced as non-reinforced. For the reasons outlined above, we anticipated control rats would be able to solve this discrimination but that perirhinal cortex lesioned rats would exhibit some difficulty. Such a pattern of results would confirm behavioral efficacy of the lesions of these rats and allow a firmer interpretation of the habituation findings of Experiments 1-3.

Method

Subjects, Apparatus & Stimuli

The subjects were those used in Experiments 1-3. After Experiment 3 ended, rats were given two-weeks' free access to both food and water in their home cages to promote recovery from weight loss before beginning Experiment 4. Throughout running of Experiment 4, rats ordinarily resided in their home cages with food and water freely available. Discrimination training occurred in a rectangular, grey, fiberglass tank (100 cm long, 62 cm wide and 62 cm deep). A gray plastic partition wall (62 cm high and 46 cm long) protruded perpendicularly from the middle of one of the 62-cm-wide walls (*stimulus wall*). The partition ran vertically to create left- and right-hand side locations for the two compound stimuli on the stimulus wall (see below). A transparent plastic escape platform (4 mm thick, 11 cm long and 9 cm wide) could be located centrally on either the left or right hand locations of the stimulus wall with its 11-cm edge horizontal and in contact with it. The tank was filled to a depth of 32 cm with water maintained at 25°C using a 300-W aquarium heater (AquaLine, UK) and made opaque by adding 35 mL of a non-toxic, white emulsion (Opacifier, Chesham Chemicals, Harrow, UK). The tank was emptied, cleaned and replenished each day. The escape platform was 2 cm below the surface of the opaque water and so was ordinarily invisible. The left and right side locations of the stimulus wall were designed to allow attachment of paper sheets, which were used for stimulus presentation. Each sheet was waterproofed by enveloping it in a transparent plastic lamination sheet. Sheets were centrally located on each stimulus wall location and were above the water line. All of the sheets had a pair of stimulus elements printed on them, which were 14 cm wide, 28 cm high. The pair of stimulus elements was positioned side by side making the compound stimulus 28 cm wide, 28 cm high). There were a total of four stimulus elements: a black rectangle (B); a series of 2.5-cm, horizontal black and white stripes (S); a white rectangle (W); and a white ellipse (14 cm wide and 24 cm high), positioned centrally on a black rectangle (E). Stimulus elements were arranged to create four compounds: B|S (i.e., B to the left of S); S|B; W|E; and E|W, see Fig. 5, bottom left panel). Stimulus elements were not arranged to make any other compounds. The compound stimuli B|S, S|B, W|E and E|W could be presented on the left or right locations of the stimulus wall. To reduce the possibility of discriminative performance being controlled by cues created by a rat's contact with the stimuli (e.g., olfactory cues), multiple copies were used which were cleaned between successive rats. Furthermore, the counterbalanced arrangement of particular compound stimuli to experimental condition rendered impossible solution by such means (see section below).

Procedure

Training occurred in three stages: platform pretraining; discrimination training; and, transfer test. Stage 1 (*platform pretraining*) was designed simply to accustom rats to the apparatus and to effective use of the submerged escape platform and none of the stimuli, B, W, S or E elements were presented. During platform pretraining, rats were individually placed on the escape platform and from time to time lowered into the water close to the escape platform, where they were allowed to return immediately to the platform. Over successive trials, rats were placed in the water increasing distances from the escape platform until they were efficiently swimming the full length of the water tank. After that, rats were lowered into the

tank from the wall opposite the stimulus wall. Positioning of the escape platform to left and right locations of the stimulus wall occurred randomly with the constraints that there were ten trials in each location and that no more than three trials in either position could occur in succession. Rats could swim in any area of the tank but they were motivated to find the escape platform to avoid the water. Rats were permitted to remain in the tank until they found the escape platform where they remained for 10 s before immediate removal from the tank and placement in a dry box. 20 s later the next trial began. There were twenty trials in each of a pair of platform-pretraining sessions, which were followed on the next day by stage-2 training (*discrimination training*). Discrimination training (see Fig. 5, right panel) was identical to platform pretraining except that now two of the compound stimuli, were presented on the water tank's stimulus wall. For all rats, the compound B|S was presented with compound W|E. The location of each compound on the stimulus wall was randomly determined with the constraints that: (a) there were ten trials with B|S on the left of the stimulus wall and W|E on the right (B|S versus W|E) and that there were also ten trials with W|E on the left and B|S on the right of the stimulus wall (W|E versus B|S); (b) there were no more than two B|S versus W|E trials or W|E versus B|S trials in succession. For half of the rats in group Sham and half of the rats in group PeRh, the escape platform was located adjacent to B|S, but never adjacent to W|E; for the remainder, the reinforcement contingencies were reversed. A response was recorded as incorrect if a rat's snout came within 20 cm of the stimulus-wall partition lacking the escape platform, though the rat was still permitted to locate the platform by swimming around to the other side of the water-tank. Twenty trials were given in each of four discrimination-training sessions.

Stage 3 (*transfer test*) consisted of a single test session beginning the day after the fourth discrimination training session and in which two previously reinforced compounds were again used. For half of the rats, the transfer test consisted only of the compound B|S versus S|B; for the remainder, it consisted only of the compound W|E versus E|W (see Fig. 5, right panel). Subgroups created by stage-2 counterbalancing were represented equally in these alternative transfer-test treatments. This created four counterbalanced subgroups ($n_s = 4$), in which rats from group Sham and group Peri were equally represented. Again, choice of B|S and W|E only were reinforced (S|B and E|W were never reinforced) and compounds appeared on left and right positions on the stimulus wall (e.g., B|S versus S|B and S|B versus B|S) in randomly determined sequence with no more than two types of trial in sequence and with eight of each trial type.

Results & Discussion

Discrimination

Rats in both groups readily learned to locate the escape platform during platform pretraining (stage 1) in which none of the stimulus elements was presented. Results from the four sessions of discrimination training (stage 2), in which B|S versus W|E trials were given, are summarized in the left panel of Fig. 6 and are presented as proportion correct, where 0.5 represents chance performance and 1.0 represents perfect performance. Both groups' performance began at about chance (0.5) and rose to almost perfect performance (1.0) by the fourth session of discrimination training. There was no apparent group difference in the rate

or in the level of the two groups' performance. ANOVA revealed a significant effect of session, $F(3,42) = 72.34$; $p < .001$, but no significant effect of group nor interaction between these factors ($F_s < 1$). Polynomial trend tests revealed linear, quadratic and cubic trends, smallest $F(1, 14) = 11.05$; $p < .005$. The observation that perirhinal cortex lesions leave a simultaneous discrimination intact is in keeping with other reports with this procedure (e.g., Davis et al., 2007; see also Eacott, 2001).

The data of central importance are those from the transfer test (stage 3) of the experiment, summarized in the right panel of Fig. 6. Here, rats received training in which it was necessary to discriminate one of the stage-2 compounds from its mirror-image version; that is, choice was between either B|S versus S|B or, for other rats, between W|E versus E|W. Rats' performance dropped from the level attained during discrimination training; but group PeRh's performance was more severely disrupted than group Sham's, demonstrating a specific difficulty in discriminating the transfer-test compounds from their reversed versions. That description of the summary data was confirmed by ANOVA, which revealed main effects of stage (i.e., the fourth session of discrimination training versus the transfer test), $F(1, 14) = 63.39$; $p < .001$; group, $F(1, 14) = 8.94$; $p < .010$ and an interaction between those factors, $F(1, 14) = 4.68$; $p < .049$. The source of this interaction was established using simple main effects analysis with a common error term and revealed a significant group difference during the transfer test, $F(1, 14) = 13.39$; $p < .010$, but no group difference on session 4 of discrimination training, $F < 1$. One-sample tests confirmed that group Sham's transfer-test performance was better than chance, $t(7) = 4.25$; $p < .004$, but that group PeRh's was not, $t < 1$. It seems inevitable that some performance decrement should be seen in the transfer from stage-2 to transfer-test, if only the result of generalization decrement (e.g., Hull, 1945). In addition, if we assume that stage-2 performance was governed by two processes – approach strength toward B|S and avoidance of W|E – the replacement of, say, W|E with S|B during the transfer test, would leave only the B|S's approach strength available to guide performance. Performance may undergo further disruption because, in this example, S|B is to be avoided but it is composed of identical stimulus elements to the compound B|S, which must be approached. Thus, good transfer-test performance is fully reliant on some means of discriminating compound stimuli from their mirror images; such discriminations have been termed *structural* discriminations (see, e.g., George, Ward-Robinson & Pearce, 2001; Davies, et al., 2007) and may be regarded as a special form of configural discrimination. There was, of course, no requirement for rats to encode the spatial arrangement of elements of the stage-2 compounds – rats' performance could have been based on, for example, approach of B and/or S, with no sensitivity to the B|S arrangement – but the fact that group Sham's performance was, to some extent, maintained during the transfer test implies that structural information *was* encoded and that this encoding was at best limited in group PeRh.

It is noteworthy that some *peripheral* process associated with perirhinal damage could contribute to group PeRh's poor performance during stage-3 (transfer test). And once such a position is taken, there is no necessity to interpret the current experiment's results as the demonstration of a lesion-induced deficit of discrimination learning. For example, lesion-induced hyperactivity could reduce performance by affecting rats' view of the compound

stimuli, making them choose with inappropriately haste; or perhaps making rats prone to fall from the escape platform (rendering the reinforcer less effective). Informal analysis of the rats' behavior during experimentation failed to indicate this: there was no obvious overt behavioral difference between rats. Furthermore, peripheral lesion effects would also produce a deficit during stage-2 (discrimination training), but none was seen here (despite there being a greater opportunity to see such a difference). Our conclusion, therefore, is that the lesion affected discrimination learning and not some peripheral process.

General Discussion

Comparator theories, such as Konorski's (1967; see also Wagner, 1979) have in common their assumption that incoming sensory information is compared to a set of central mnemonic representations. In these terms, a new stimulus – not yet represented – elicits an unconditioned response that habituates as a result of the formation of its representation. Variants of this position have been expanded to include the suggestion that the representation is cortical (e.g., Sokolov, 1963) and we suggested that the perirhinal cortex might be a suitable candidate for storage of such a representation. One reason for that supposition is that the perirhinal cortex is in receipt of information from multiple senses (see, e.g., Burwell & Amaral, 1998; Shi & Cassell, 1997) and so would be well-placed for the formation of stimulus representations. But more direct evidence is provided by the finding that manipulations of the perirhinal cortex attenuate performance of the spontaneous object-recognition procedure (see, e.g., Aggleton, Keen, Warburton & Bussey (1997; Albasser, et al., 2009; Warburton et al., 2003; Winters & Bussey, 2005; Winters, Saksida & Bussey, 2008). In such tasks, rats are presented simultaneously with a pair of objects, one to which they have been previously exposed, the other being novel. Rats are found to bias their exploration to the novel object – a result indicating habituation of exploratory reaction elicited by the familiar object. The fact that perirhinal cortex manipulations attenuate that habituation of exploration in spontaneous object-recognition raises the possibility that the perirhinal cortex plays a more general role in habituation. Such a finding would be significant, not only in its identification of a neural substrate of habituation, but in that it would implicate habituation as a mechanism of object recognition. However, our current findings run contrary to that position as we were unable to find any influence of perirhinal lesions in habituation procedures using, visual, auditory or gustatory stimuli. But the perirhinal lesions were not without behavioral effect as there was a disruption to the transfer test of a visual discrimination, indicative of a failure to learn spontaneously the configural properties of the visual stimuli.

What then is the basis for the difference in the effects of perirhinal lesions on spontaneous object-recognition and in our current experiments? All three of our experiments used habituation of suppression (of operant responding for food, Experiments 1 and 2 and of drinking, Experiment 3), whereas spontaneous object-recognition relies on habituation of exploration. Several authors have drawn distinctions between behavior systems (e.g., Konorski, 1967; see also, Jordan et al., 2000); and here it is possible that the perirhinal cortex is necessary for the habituation of exploratory behavior but not for habituation of suppression. A second difference may lie in the types of stimuli used. In our habituation experiments, the stimuli might be regarded as rather simple in comparison to object stimuli

that are used in recognition experiments (see, e.g., Bartko, Winters, Cowell, Saksida & Bussey, 2007; Norman & Eacott, 2004). The perirhinal cortex is in receipt of relatively highly processed visual information, for example the ventral temporal association cortex supplies about one third of the afferents to the rat's area 36 (e.g., Burwell & Amaral, 1998). For these reasons, perhaps such complex visual object, stimuli are sensitive to perirhinal lesions but the simpler (and often non-visual) stimuli of our current experiments are not. However, in one experiment by Albasser et al. (2009), rats with lesions of the perirhinal cortex were presented with an object of the sort used in standard demonstrations of object recognition. Rather than proceeding with the standard test (comparison of exploration of the familiar object, with a novel object), Albasser et al. gave an additional three daily trials until the level of exploration had habituated to a near-negligible level. This relatively fine-grained examination of habituation of exploratory behavior yielded no difference between the lesioned rats and their controls – a pattern of results demonstrating that the insensitivity of perirhinal lesions on habituation tasks is not restricted to the suppression response used in the current report and may well extend to the class of stimuli that show impairments on tests of object recognition.

We described habituation as a process in which current sense information is compared to a centrally stored representation (e.g., Konorski, 1967; Sokolov, 1963; see also Wagner, 1979) but some authors emphasise a simpler model of habituation, expressed in terms of a decline in the transmission between a stimulus-and-response (S-R) pathway (see, e.g., Gray, 1975; Horn, 1969; Mackintosh, 1987). The S-R mechanism is attractive, not only because of its parsimony but because it appears to be essential to accommodate demonstrations of habituation in invertebrate organisms (see, e.g., Pinsker, et al., 1970; Tomsic, et al., 1998; see also, Jennings, 1906; Wood, 1973), appearing to lack the neural circuitry one would assume the central representation requires. Of course, the presence of a S-R mechanism need not preclude an additional comparator mechanism in vertebrate organisms, having relatively complex central nervous systems.

If we allow that both comparator and S-R processes underlie habituation (at least for certain vertebrates; but see Horn, 1967; Mackintosh, 1987), we go some way to providing an account of the observation that rats with perirhinal lesion show normal spontaneous object recognition when the interval between the sample presentation and the choice test is rather short (typically less than 24 hours) but fail at longer intervals (see, e.g., Ennaceur, et al., 1996; Norman & Eacott, 2004; but see, Mumby, et al., 2007). Thus, a spontaneous object recognition experiment with a short sample-choice test interval might invoke a short-term transmission fatigue in the S-R pathway, the stimulus here being a pattern of features corresponding to the familiar junk object; the response being the tendency to approach or investigate. Evidence from habituation experiments in single-unit electrophysiological experiments probably represents a purely S-R process, which recovers (at least to some extent) after a rest period (e.g., Mackintosh, 1987; Thompson & Spencer, 1966); but there is no such necessity to assume such short-term recovery for the comparator mechanism. Thus, normal rats' performance will be governed by both mechanisms when appropriately short sample-choice intervals are employed. But with longer intervals, the S-R pathway's transmission will be refreshed (i.e., the familiar object will elicit approach behaviour again); however, the comparator mechanism will remain effective and capable of suppressing

approach behaviour in normal rats. Perirhinal rats, however, may not be affected at shorter intervals (the effect of S-R fatigue being sufficient to produce normal performance), but at long intervals where only the absent comparator may be effective, their performance is attenuated. We noted above that Albasser, et al. (2009) found rats' exploration of a junk object habituated over four trials that were separated by 24-hour interval and that this habituation was not dependent on the integrity of the perirhinal cortex. However, although S-R recovery occurs some time after habituation, it is incomplete – there is at least some, longer-term S-R habituation process (see, e.g., Thompson & Spencer, 1966).

Our characterization above of object recognition being sensitive to perirhinal damage only at sample-choice intervals of more than 24 hours is somewhat inaccurate, with deficits having been reported at intervals as short as 10 minutes (e.g., Norman & Eacott, 2004) and intact performance at 24-hour intervals (Mumby et al., 2007). It is, of course, hazardous to take such cross experiment comparisons at face value because many differences exist between object recognition experiments, which may affect lesion sensitivity (e.g., relatively extensive sample exposure or use of relatively salient object, may compensate for a genuine lesion deficit). That is, they should not be taken to disconfirm the observation that lesion sensitivity is most marked at longer intervals which is legitimately assessed only within experiment (e.g., Norman & Eacott; Ennaceur et al., 1996; Mumby et al.). And, of course, it is this *general* time-related feature of the sensitivity of perirhinal lesions to intervals (rather than any *specific* interval, per se) that is important for analysis suggested above.

Wagner (e.g., Wagner, 1979) suggested a general model that naturally anticipates distinctions between long- and short-term habituation (e.g., Davis, 1970; Sanderson, Good, Skelton, Sprengel, Seeburg, Rawlins et al., 2009; Tomsic, et al., 1998; but see Mackintosh, 1987). Here, the initial presentation of a stimulus will result in its representation entering a primary activity state (*A1*), which may generate relatively strong unconditioned responding. Iterative stimulation will come to elicit weaker unconditioned responding (i.e., short-term habituation) because the stimulus representation becomes *primed* (*self-generated* priming) into its secondary activity state (*A2*), able to support only rather weak unconditioned responding. It is only following a period of inactivity (*I*) that the stimulus can enter its *A1* state of activity in which it can, again, provoke strong unconditioned responding (dishabituation). The apparatus cues (context) may be able to enter into association with the primary activity state representation, such that future placement in that context, again, prime the representation into its secondary activity state (*retrieval-generated* priming) capable of only weakly provoking the unconditioned response (see, e.g., Davis, 1970; Jordan et al., 2000; Sanderson, et al., 2009; Tomsic et al., 1998; but see Hall & Channell, 1985; Hall & Honey, 1989).

The failure of rats with perirhinal cortex lesions to perform normally at spontaneous object recognition with longer interstimulus intervals (e.g., Ennaceur, et al., 1996; Mumby, et al., 2007; Norman & Eacott, 2004) can be accommodated by Wagner's (e.g., Wagner, 1979) model if it is assumed that that some aspect of those rats' learning about contexts is impaired. We note that rats with perirhinal lesions have been reported to show attenuated discrimination between contextual stimuli (see, e.g., Bucci, Phillips & Burwell, 2000; Bucci, Saddoris & Burwell, 2002; Howse, Squires, Martin & Skinner, 2003; see also, Norman &

Eacott, 2005), which may prevent the context-object associations required by Wagner's account of long-term habituation (i.e., retrieval-generated priming). But our current findings appear to speak against this analysis because perirhinal cortex lesions did not, in fact, attenuate long-term habituation. However, it is unlikely that the contexts were particularly effective in producing retrieval-generated priming because of rats' familiarity with the contexts before presentation of the stimuli, a process analogous to latent inhibition (see, e.g., Wagner, 1979; McLaren & Mackintosh, 2000). In Experiments 1 and 2, rats were familiar with the Skinner boxes before the presentation of visual or auditory stimuli, because it was necessary to first (Experiment 1) establish the instrumental response. And the context in Experiment 3 was the home cage, in which rats spent most of their lives. In each case, rats' relative familiarity with the context would be predicted by Wagner to minimise the extent of the context's primary activity, necessary for the establishment of the excitatory association between the context and the stimulus. Thus, if the procedure diminished a component of habituation reliant on contextual processing (and relied upon another process), the contribution of any lesion-induced deficit in context processing (e.g., Bucci, Phillips & Burwell, 2000; Bucci, Saddoris & Burwell, 2002; Howse, Squires, Martin & Skinner, 2003) would be expected to be minimal.

We set out to assess the suggestion that the perirhinal cortex might play a role in habituation of unconditioned responding. Our reason for interest in that brain region was because of its involvement in procedurally similar object recognition tasks (e.g., Albasser, et al., 2009; Liu & Bilkey, 2001; Mumby, et al., 2007; Warburton, et al., 2003; Winters, et al., 2004), which are explicable in terms of a comparator model, such as that described for habituation (e.g., Sokolov, 1963; Konorski, 1967; see also, Fernández & Tendolkar, 2006). To that end, we investigated the role of the perirhinal cortex on habituation using a variety of different stimuli, all having anatomical representation in the perirhinal cortex (see, e.g., Burwell, 2001; Burwell & Amaral, 1998), and took steps to provide sensitive measurements of changes in behavior by examination within individual trials. Despite the broad scope of these experiments, we were unsuccessful in detecting involvement of the perirhinal cortex in any of the experiments, despite the lesion's effect in a visual discrimination problem. Our results, therefore, imply that different processes operate in the normal operation of habituation and other forms of visual discrimination, though they do not permit us to indicate what they might be.

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Fig. 1.

Histological reconstructions for Experiments 1 – 4. The maximum (gray) and minimum (black) extents of perirhinal lesions at coronal sections, taken through the rostro-caudal axis of the brain. Sections are based on *The Rat Brain in Stereotaxic Coordinates*, by G. Paxinos and C. Watson, San Diego, CA: Academic Press. Copyright 2005. Adapted with permission from Elsevier. Sections correspond to sections –2.80 mm, –3.80 mm, –4.80 mm, and –5.80 mm relative to bregma.

Fig. 2.

Means, and one standard error of each mean, of the data from Experiment 1. Group Sham and group PeRh responded on an instrumental baseline for food reinforcement and a visual stimulus (e.g., operation of a lamp for one minute) were presented twice in each of four sessions. The left panel summarizes responding during those eight trials. Data are ratios based on the response rates during each trial and the response rate during the one-minute period that immediately preceded it. The right panel summarizes responding during only the first trial, which has been split into four 15-s periods. Ratios less than zero indicate that responding during the light was suppressed relative to the baseline rate (see text for details).

Fig. 3.

Means, and one standard error of each mean, of the data from Experiment 2. Group Sham and group PeRh responded on an instrumental baseline for food reinforcement and an auditory stimulus was presented twice in each of two sessions. The left panel summarizes responding during those four trials. Data are ratios based on the response rates during each trial and the response rate during the one-minute period that immediately preceded it. The right panel summarizes responding during only the first trial, which has been split into four

15-s periods. Ratios less than zero indicate that responding during the light was suppressed relative to the baseline rate (see text for details).

Fig. 4.

Means, and one standard error of each mean, of the data from Experiment 3. Group Sham and group PeRh were offered an aqueous flavored solution for one hour over four daily sessions. The left panel summarizes data from the first 15 minutes of the four sessions. The right panel summarizes data from the remaining 45 minutes of each session (see text for details).

Fig. 5. Water tank apparatus (top left), compound stimuli (bottom left) and experimental design (right) of Experiment 4. Individually run rats were lowered into the tank's opaque water from the start point where they could swim toward an escape platform, located below the stimulus wall. (*Note* – the escape platform is visible in the figure but was submerged and, therefore, invisible in the actual experiment; see text for full details). Compound stimuli depicted are B|S versus W|E and appear as they might on the water tank's stimulus wall during the transfer test. Compounds could be presented in the alternative position on the stimulus wall (i.e., W|E versus B|S) and could be presented with their mirror image forms (i.e., B|S versus S|B and W|E versus E|W), which occurred only during the transfer test. Rats in group Sham and group PeRh received training on one of the four discriminations shown, where the check (✓) indicates the compound adjacent to the escape platform; the cross (✗) indicates the compound without the escape platform. The first pair of columns indicate the stimulus wall's display during discrimination training; the second pair of columns indicate the stimulus wall's display during the transfer test. Notice that each compound appears on the left and right positions of the stimulus wall.

Fig. 6.

Means, and one standard error of each mean, of the data from Experiment 4. Data are proportions correct in which 0.5 represents chance performance and 1.0 represents perfect performance. Group Sham and group PeRh were trained to swim toward black and white compound stimuli in a water-filled tank. Pairs of compounds composed of elements, B, S, W and E were presented on each trial. Correct choice was reinforced by access to an invisible escape platform. The graph on the left of the figure represents acquisition of discrimination training (stage 2) having B|W versus W|E, and W|E versus B|S trials. The histogram on the right of the figure represents rats' performance during the transfer test in which mirror-

image forms of the compounds (e.g., B|W versus W|B) from the preliminary discrimination were introduced (see *Method* for full details).