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Conditioned inhibition in the Spatial domain in Humans and Rats

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Abstract

Spatial learning has been shown to follow associative rules by demonstrations of blocking and overshadowing in both watermazes with rats and virtual watermazes with humans. To examine whether Conditioned Inhibition (CI) can also be demonstrated in a real and virtual watermaze task, two studies were run, one with rats and one with humans. In separate training trials, Beacons A and B marked the position of a platform in quadrant X of circular watermaze (AX+/BX+). In subsequent inhibitory training trials, Beacon A was placed in quadrant Y with no platform present (AY-). To test for any CI of Y, in two probe trials B was suspended above either quadrant Y (BY) or novel quadrant Z (BZ). Time spent under B was recorded in both trials. In both animal and human studies, during no platform probe trials, latencies to reach Beacon B were longer and less time was spent under the beacon when it was suspended in quadrant Y, where inhibitory training had previously taken place (AY- trials), than when it was hung in the novel quadrant Z. Results suggest that quadrant Y had become a conditioned inhibitor strengthening claims that learning in the spatial domain follows the rules of associative models.

Conditioned inhibition in the Spatial domain in Humans and Rats

Blocking one element of a compound from forming an association with an unconditioned stimulus by pre-conditioning the other element is predicted by associative learning models such as the Rescorla-Wagner model (1972). The Rescorla-Wagner model assumes that elements in a compound compete to gain associative strength when paired with an unconditioned stimulus. If the preconditioned element has already gained all of the associative strength then the second element cannot gain any strength. Blocking has been demonstrated in the spatial domain both with rats in watermazes (Redhead, Roberts, Good, & Pearce, 1997) and with human participants performing equivalent computer generated watermaze tasks (Redhead, Hamilton, Parker, Chan, & Allison, 2013; Wilson & Alexander, 2008). Chamizo (2003) and Miller and Shetleworth (2007) have suggested that such demonstrations illustrate that learning in a spatial domain is governed by a general process relying on elements competing for associative strength which is predicted by associative models like the Rescorla-Wagner model. However, such blocking of elements might be equally explained by attentional processes, whereby the participants only attend to the preconditioned element. A phenomenon within associative learning more difficult to explain via attentional processes is conditioned inhibition (CI). The aim of this paper is to test whether CI can be demonstrated in a spatial domain with both rats and humans and in a way that can only be predicted by associative models.

In a typical CI experiment (Rescorla, 1969), a stimulus (Y) is paired with a second stimulus which has been previously associated with a US (A+). In the presence of Y, A is no longer associated with the US (AY-), leading to Y becoming a conditioned inhibitor (Mackintosh, 1983). CI of Y is demonstrated by summation and retardation tests ((Rescorla, 1969). In a summation test, a reduced response to a second conditioned stimulus (B+) in the presence of Y (BY) compared to the response to B in the presence of a novel stimulus Z (BZ) illustrates conditioned inhibition. Summation effects have been demonstrated with various species and methods, for example with key-pecking in pigeons (Wessels, 1973) and with food aversion in rats (Taukulis & Revusky, 1975). In a retardation test, prior inhibitory conditioning of a CS retards subsequent excitatory conditioning to that CS. Therefore, conditioning to Y+ should be slower compared to excitatory conditioning to a previously novel stimulus. Again retardation has been demonstrated with various species and methods, for example with salivation conditioning in dogs (Konorski & Szwejkowska, 1952) and with eyelid conditioning in rabbits (Marchant, Mis, & Moore, 1972).

However, there have been mixed results from summation and retardation tests following CI training in humans. For example, Artigas, Chamizo and Peris (2001) demonstrated both summation and retardation following conditioned inhibition of auditory cues, and Thurston and Cassaday (2015) demonstrated summation and retardation in a CI paradigm with visual cues from the International Affective Picture System (IAPS). Grillon and Ameli (2001), on the other hand, found no evidence of summation following CI training of fear-potentiated startle and skin conductance in humans.

In the current studies, CI was tested in a spatial domain by placing a beacon (A) above the platform in quadrant X of a circular watermaze (AX+). In subsequent inhibitory training trials, Beacon A was placed in quadrant Y with no platform present (AY-). To test for any CI of Y, another beacon B, which had previously only been used in trials with the platform present (BX+), was suspended above either quadrant Y (BY) or novel quadrant Z (BZ) where the platform had never been placed during training. If quadrant Y had become inhibitory it would be expected that time to approach Beacon B would be longer when it was above quadrant Y compared to the novel quadrant Z. In subsequent retardation tests the rats and humans were trained to locate a hidden platform in either quadrant Y or the novel quadrant Z. Again, if Y had become a conditioned inhibitor it would be expected that the number of trials to learn the platform is in quadrant Y would be more than if the platform was in the novel quadrant Z.

Sansa, Rodrigo, Santamarı´a, Manteiga, and Chamizo (2009) and Horne and Pearce (2010) have demonstrated CI within the watermaze using rats, suggesting that, at least with rats, CI in the spatial domain is governed by associative processes. There are other ways to explain CI without recourse to the competitive associative learning predicted by the Rescorla-Wagner model (1975). For example Amsel and Roussel (1952) suggested that an inhibitory stimulus such as Y, rather than becoming inhibitory in order to counter the associative strength of A, might simply induce avoidant behavior due to the omission of a predicted appetitive reinforcer. One way to test whether the CI seen in Sansa et al. (2009) and in Horne and Pearce (2010) was due simply to avoidant behavior would be to present a trial with Y alone following the CI training. If Y is inhibitory due to the associative strength of A, as predicted by the Rescorla-Wagner model, then in the absence of A there should be no avoidance of Y. The current paper will seek to extend the findings of Sansa et al (2009) and Horne and Pearce (2010) by presenting Y alone following CI training.

Experiment 1 examined CI with rats in a watermaze similar to those used by Sansa et al (2009) and Horne and Pearce (2010). The current study also differed from the previous studies as to the type of stimuli that were trained as conditioned inhibitors. In the previous studies, specific landmarks were only present on trials with no platform and thus became inhibitory. In the current study, it was the location of the landmark which became inhibitory, as no platform was present on trials when the landmark was in the SW corner. Experiment 2 will further seek to extend the generality of processes governing CI in the spatial domain by testing for CI in humans with a virtual watermaze.

Experiment 1

*Method*

*Participants*

Twenty four male Lister-Hooded rats weighing 248-361 g were used in this study, 12 in the novel group, 12 in the inhibitory group. They were approximately 4 months old at the start of this study. Animals were housed in cages of 3-6 with a 12/12 hr light/dark cycle and were given free access to food and water. Initially the rats were handled for approximately 1 hr per cage over 6 days to minimise the effects of handling stress on performance. Experiments were carried out in accordance with the current British Home Office guidelines, and with consent of the University of Southampton Bioethics Committee. All attempts were made to reduce the number of animals and the degree of suffering.

*Materials and apparatus*

A watermaze was used to assess spatial learning. A white circular plastic pool 2 m in diameter was placed in the centre of a 4 m x 6 m room with white walls. The pool was filled with water, at 23ºC ± 1ºC, and 1 litre of milk (to make it opaque) to a depth of approximately 270 mm. The water and milk were changed daily. A 10 x 10 cm circular white platform was used which was always 2.5 cm below the water. The pool was illuminated from above by two strip lights, and visual cues in the room were kept constant throughout the experiment. In the centre of the South wall at a height 2 m, there was a predominantly blue poster (0.5m x 1m). In the corner of the north wall was the green door to the room. In the west corner, a 1.8 m x 0.6 m screen was placed 1 m from the pool. Along the length of the East wall was a shelf at a height of 1.5 m. A video camera (Hi-resolution B/W CCD camera, Sanyo) with a wide-angle lens (1.8-3.6 mm, 1:1:6, 1/3”, CS, Computer) was fixed 1.5 m above the centre of the pool. During trials, the experimenter sat behind a screen in the south east corner of the room and observed the activity of the rat on a television monitor. For test trials, the video image of the rat’s movements was digitized with a Dazzle digital video creator, stored in MPEG 1 format, and then analysed with Ethovision video tracking software (version 3.0, Noldus).

Beacons were hung from the ceiling 30 cm above the water surface using black fishing wire. Beacon A was a red and yellow spotted ball (10 cm diameter) and Beacon B was a black and white striped tube (5 cm diameter, 10 cm in length).

*Procedure*

Training and testing took place over 20 days. Rats were transported to the test room four at a time in a box that was placed on the floor in the northwest (NW) corner of the room. Four trials were given on days 1-5. The platform, with Beacon A suspended above, was placed in one of two different locations, which were on the midpoints of the radii pointing NW, through quadrant X (trial AX+), and pointing SE through quadrant W (trial AW+). If a rat failed to find the platform within 60 s, the experimenter placed a thumb about 5 cm in front of the rat’s nose and guided it to the platform. Rats were allowed to remain on the platform for 30 s before they were removed from the pool. They were then either returned to their home cage after being dried, or placed on a towel on a chair for 60 s before being returned to the pool for the next trial. The platform was moved from trial to trial. The choice of locations was random with the constraint that the same position was used no more than three times in any day, and that each position was used 10 times throughout days 1-5. The animals were released from the edge of the pool at four positions that corresponded to the principal points of the compass. Each position was used once in a day in a randomly determined sequence. Throughout the experiment, escape latencies were recorded by the experimenter using a stop watch, which was started when the rat was released from the edge of the pool and stopped when the rat started to climb on the platform. On day 6, two AX+ trials and one AW+ trial were run. In addition, there was one AY- trial. On these trials the platform was removed from the pool and Beacon A was placed above the midpoint of the radius pointing SW through quadrant Y (AY- trials). During AY- trials, the rats were allowed to swim for 60 s and were then removed from the pool. Time spent beneath Beacon A within a 15 cm radius of the midpoint of the radius pointing SW (platform area) was recorded. On days 7-9, one AX+, one AW+ and two AY- trials were run. On days 10-13, an additional trial of BX+ and one trial of BW+ were run. On these trials, Beacon B was placed above the platform in either quadrant W (BW+) or quadrant X (BX+ trials). On day 14, one AX+ and one BX+ trial were run for all rats. On the remaining trial, the platform was removed from the pool and Beacon B was placed above quadrant Y (BY- trials) for half the rats. For the other half of the rats, the midpoint of the radius pointed NE through quadrant Z (BZ- trials). Latency to cross the platform area and time spent in the platform area were recorded. On day 15, the previous trials were run again, except the rats which had Beacon B above quadrant Y on day 14 had Beacon B placed above quadrant Z, and the rats which previously had the Beacon above Z had the beacon now placed above Y. On day 16, no beacons or platforms were placed in the pool and the time spent in the four platform positions in quadrants Y, X, W and Z was recorded. On days 17-20, the rats were split equally on a random basis into each of two groups, Group Novel and Group Inhibit. For Group Novel, the platform was placed on the midpoint of the radius pointing NE through quadrant Z (Z+ trials), and for Group Inhibit the platform was placed on the midpoint of the radius pointing SW through quadrant Y (Y+ trials). Latency to locate the platform on each trial was recorded.

Table 1

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Days 1-5 | Day 6 | Days 7-9 | Days 10-13 | Days 14-15 | Day 16 | Days 17-20 |
| 2AX+ 2AW+ | 2AX+ AW+  AY- | AX+  AW+  2AY- | AX+  AW+  2AY-  BX+  BW+ | AX+  BX+  BY-/ BZ- | W-  X-  Y-  Z- | Y+/Z+ |

*Results*

*Training trials*

Latencies to find the platform under Beacon A and B dropped over training (Figure 2). Time spent under Beacon A when there was no platform present in the inhibitory trials (AY-) also declined during training. This impression was confirmed in a series of Analyses of Variance (ANOVA).

A mixed design Group (Novel v Inhibitory) x Pool Quadrant (X v W) x Trial ANOVA was performed on the latencies to reach the platform under Beacon A in quadrants X and W. The effect of group was not significant, *F*(1, 22) = 1.373, *p* = 0.254, *ηp*2 = .059, nor was the effect of pool quadrant, *F*<1, but the effect of trial was significant, *F*(16, 352)= 30.82, *p* < 0.000, *ηp*2 = .583, with escape latencies decreasing over trials.

A mixed design Group (Novel v Inhibitory) x Pool Quadrant (X v W) x Trial ANOVA was performed on the latencies to reach the platform under Beacon B in quadrants X and W. The effect of group was not significant, *F*(1, 22) =2.09, *p* = 0.162, *ηp*2 = .087, nor was the effect of pool quadrant, *F*<1, but the effect of trial was significant, *F*(2, 44) = 10.94, *p* < 0.000, *ηp*2 = .333, with escape latencies decreasing over trials.

A mixed design Group (Novel v Inhibitory) x Trial ANOVA was performed on the time spent under Beacon A when no platform was present in quadrant Y. The effect of group was not significant, *F*(1, 22)=2.06 *p* = 0.165, *ηp*2 = .086 , but the effect of trial was significant, *F*(12, 264)= 22.09, *p* < 0.000, *ηp*2 = .501, with time spent under the beacon decreasing over trials.

*Summation BY and BZ Test trials*

More time was spent under Beacon B in the Novel quadrant (Z) than in the Inhibitory quadrant (Y) (Figure 3: Left hand Panel). Latencies to pass under Beacon B in quadrant Z were shorter than to pass under Beacon B in quadrant Y (Figure 3: Right hand Panel). These impressions were confirmed by a series of ANOVAs.

A mixed design Group (Novel v Inhibitory) x Trial Type (BY v BZ) ANOVA was performed on the time spent under Beacon B when no platform was present. The effect of group was not significant, *F*<1, but the effect of trial type was significant, *F*(1, 22)= 109.2, *p* < 0.000, *ηp*2 = .832, with more time spent under Beacon B in BZ trials.

A mixed design Group (Novel v Inhibitory) x Trial Type (BY v BZ) ANOVA was performed on the latency to cross the platform area under Beacon B. The effect of group was not significant, *F*<1, but the effect of trial type was significant, *F*(1, 22)= 24.63, *p* < 0.000, *ηp*2 = .528, with shorter latencies to pass under Beacon B in BZ trials than in BY trials.

*Empty Pool trials*

In order to test whether the greater time spent under the beacon when it was above quadrant Z as opposed to quadrant Y was due to avoidance of Y quadrant in a manner described by Amsel and Roussel (1952) as opposed to the establishment of Conditioned Inhibition via a process described by the Rescorla-Wagner model (1975) or due to an unconditioned preference for quadrant Z over the other quadrants, the time spent in the pool without any beacons was measured. There was no difference in the time spent in the four platform areas (Figure 4). These impressions were confirmed by an ANOVA.

A mixed design Group (Novel v Inhibitory) x Platform Area (X v Y v Z v W) ANOVA was performed on the time spent in the four platform areas. Both the effect of group, *F*(1, 22) = 4.12 *p* = 0.055, *ηp*2 = .158 , and the effect of platform area were not significant, *F*<1, illustrating that rats did not prefer one quadrant over another in the absence of the beacon. This observation suggests that the presence of Beacon A is required for the inhibitory effect of Y to be evident and fits best with the predictions of the Rescorla and Wagner model.

*Retardation trials*

After the initial trial the escape latencies for Group Inhibitory were longer than for Group Novel (Figure 5), suggesting that the inhibitory strength of Y retarded the acquisition of the position of the platform when it was in the Y quadrant. A mixed design Group (Novel v Inhibitory) x Trial ANOVA was performed on time to reach the platform on retardation trials. The effect of group was significant, *F*(1, 22) = 12.31, *p* = 0.002, *ηp*2 = .359, with escape latencies for Group Novel being shorter than those for Group Inhibitory. The effect of trial was also significant, *F*(5, 110) = 40.72, *p* < 0.000, *ηp*2 = .649, with the escape latencies for both groups decreasing over trials.

The results supported those of Sansa et al (2007) and Horne and Pearce (2010) in that Y became inhibitory, passing both the summation and retardation test. However, the current findings extend those of the earlier studies, as the empty pool trial data rule out Amsel and Roussel’s (1952) explanation of the observed inhibition of B by Y as due to the rats simply avoiding the Y quadrant. The first trial of the retardation test also could be seen as evidence that the rats were not simply avoiding Y, as escape latency on the first trial was the same for both Group Novel (platform Z) and Group Inhibitory (platform in Y). It was only over subsequent trials that length of escape latencies decreased more slowly in Group Inhibitory, indicative of Y’s inhibitory strength retarding acquisition of its position in the pool. The study therefore provides further evidence of the generality of learning in the spatial domain via competitive associative learning as described by associative models such as Rescorla-Wagner (1972)

Experiment 2

There have been various demonstrations of blocking and overshadowing in the spatial domain with rats (e.g. Redhead et al., 1999) and human participants (e.g. Wilson & Alexander, 2008; Redhead & Hamilton, 2007) by different papers and even within the same series of studies (Prados et al., 2013) suggesting a generality of learning. However, there have been less consistent findings with humans in CI training. While Grillon and Ameli (2001) showed no evidence of summation or retardation following CI training, both Artigas, Chamizo, and Peris (2001) and Thurston and Cassaday (2015) successfully demonstrated summation and retardation with CI paradigms using human participants. There is also debate over whether the inhibition demonstrated is via the same mechanism within animal and human studies. For example, Mitchel and Hall (2014) suggested that there was little evidence of associative inhibition in demonstrations of perceptual learning in humans and that the effects were due mostly to a reduction in salience of the common cue. Experiment 2 will seek to provide a novel demonstration of CI in the spatial domain within humans to assess the generality of the phenomenon. Experiment 2 will also seek to rule out Amsel and Roussell’s (1952) explanation of any CI observed by including the empty pool trial, as used in Experiment 1.

*Method*

*Participants*

Participants were 24 undergraduate students (8 males and 16 females) from the University of Southampton who were allocated course credits for participation in the 20-min session. They were between the ages of 18 and 25 (*M* = 20.92*, SD* = 1.51).

*Materials and apparatus*

The study took place within a windowless research cubicle, measuring 2.4 metres in length by 1.3 metres wide, with a height of 2 metres. The cubical contained a single desktop computer. The computer used a standard Windows 7 operating system, with keyboard and mouse, placed on a 1.3-m wide desk in the centre of the rear wall. The computer was connected to three identical 15-inch LCD monitors. The monitors were placed horizontally so that the displayed image was shown continuously across all three screens.

A virtual model of a circular watermaze and the surrounding room was created for this study. This model was developed by Dr Matt Jones, University of Southampton, using 3DSMax 2012. The programme placed the participants within the environment and offered a first person perspective. A layout of the cues surrounding the pool and a view of the cues from the SE quadrant of the pool can be seen in Figure 6. The model enabled participants to freely explore the pool and view the walls of the room. The virtual environment consisted of a circular pool, 75 units in diameter, with a beige coloured wall 15 units high. It took approximately 3 s to travel the diameter of the pool. An opaque, blue pattern was used to create the surface of the pool. During training, Beacons A and B were placed 20 units above the water. Both beacons were square blocks 10 units in height, A was white and B was Black. On appetitive, trials swimming beneath the blocks would end the trial. The surrounding room consisted of four beige walls 200 by 200 units. In the centre of each wall and at a height of 100 units was a unique poster, 30 by 30 units. The posters were of a building, an aeroplane, a tree and a boat. Participants controlled their movement using the “FORWARD” “LEFT” and “RIGHT” arrow keys but could not look up or down, or interact with items within the environment. Participants were unable to use the “BACK” arrow key.

*Procedure*

Training and testing took place over a single 60-min session comprising 39 trials. Participants were led into the cubicle and asked to sit in front of the computer after which the experimenter left the room. The following instructions were given to participants via the computer screen:

In this experiment you will view a computer-generated environment on the monitor. You will be viewing the environment from a first-person perspective and you can move through the environment using the arrow keys on the keyboard (UP, LEFT, AND RIGHT). You will be placed in a circular pool of water from which you must escape by climbing onto a submerged platform. When you cross the platform you will be stopped, raised out of the water, and you will see a message saying that you have found the platform. Your goal is to locate that platform and climb onto it as quickly as possible. You will be on the platform for a few moments during which time you can scan around the pool. The screen will then fade out and you will begin another trial. You will complete several trials. On each trial you will begin facing the wall of the pool.

Press the space bar when you are ready to start.

Once participants pressed the space bar, the computer screen displayed the wall of the watermaze. On turning away from the pool wall they were able to see the rest of the pool and the walls of the room with posters at their centre. The Participants were placed at North, South, East and West points of the pool at least four times each in a randomly determined sequence. On the first four trials, the platform and Beacon A was placed twice above the midpoint of the radius pointing SE through quadrant W and twice above the midpoint of the radius pointing NW through quadrant X (trials AW+ and AX+ respectively) in a randomly determined sequence. On reaching the platform, participants were placed on top of the platform for 5 s before the screen went dark for 1 s, and participants were again placed facing the wall of the pool for the start of the next trial. If the participants had not found the platform within 60 s, then the platform became visible and a message appeared on the screen instructing participants that the platform was visible and to swim toward it. The time to reach the platform was recorded for each trial. Over trials 5-9, there were 1 AW+ trial, 1 AX+ trial and 3 AY- trials. In AY- trials, no platform was in the pool, and Beacon A was placed above the midpoint of the radius pointing SW through quadrant Y; time spent in the area under A was recorded. Over trials 10-27, there were 3 AW+ trial, 3 AX+ trial 6 AY- trials, 3 BW+ and 3 BX+ trials, when the platform and Beacon B were placed in quadrant X or W; On trial 28, Beacon B was placed above quadrant Y for half the participants and above the midpoint of the radius pointing NE through quadrant Z for the other half of the participants. Time spent under the beacon was recorded. On trials 29 and 30, participants were given AW+ and BX+ trials. On trial 31 for participants who had previously had beacon B above quadrant Y on trial 28, the beacon was now placed above quadrant Z and vice versa for the remaining participants. On trial 32, participants were placed in an empty pool and time spent in the four platform areas was recorded. Over trials 33-39, the platform was placed in quadrant Y (Group Inhibit) for half the participants and in quadrant Z (Group Novel) for the other half of the participants. Latency to find the platform was recorded.

Table 2

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Trials 1-4 | Trials 5-9 | Trials 10-27 | Trial 28 | Trials 29-30 | Trial 31 | Trial 32 | Trials 33-39 |
| 2AX+ 2AW+ | AX+  AW+  3AY- | 3 AX+  3 AW+  6 AY-  3 BX+  3 BW+ | BY-/BZ- | AW+  BX+ | BZ-/BY- | W-  X-  Y-  Z- | Y+/Z+ |

*Results*

*Training trials*

Latencies to find the platform under Beacons A and B dropped over training trials (Figure 7). Time spent under Beacon A when there was no platform present in the inhibitory trials (AY-) also declined during training. This impression was confirmed in a series ANOVAs.

A mixed design Group (Novel v Inhibitory) x Gender (Male v Female) x Pool Quadrant (X v W) x Trial ANOVA was performed on the latencies to reach the platform under Beacon A in quadrants X and W. The effect of group was not significant, *F*<1, nor was the effect of gender, *F*(1, 20) = 2.12, *p* = 0.161, *ηp*2 = .096, nor was the effect of pool quadrant, *F*(1, 20) = 2.30, *p* = 0.145, *ηp*2 = .103, but the effect of trial was significant, *F*(5, 100)= 5.14, *p* < 0.000, *ηp*2 = .204, with the length of escape latency decreasing over trials.

A mixed design Group (Novel v Inhibitory) x Gender (Male v Female) x Pool Quadrant (X v W) x Trial ANOVA was performed on the latencies to reach the platform under Beacon B in quadrants X and W. No significant effect was found of group, *F*<1, pool quadrant, *F*(1, 20) < 1, gender, *F*(1, 20)= 3.11, *p* = 0.093, *ηp*2 = .135, or beacon, F<1; however, the effect of trial was significant, *F*(2, 40)= 4.81, *p* = 0.013, *ηp*2 = .194, as was the effect of quadrant, *F*(1, 20)= 14.81, *p* = 0.013, *ηp*2 = .194 .

A mixed design Group (Novel v Inhibitory) x Gender (Male v Female) x Trial ANOVA was performed on the time spent under Beacon A when no platform was present in quadrant Y. Both the effects of group and gender were not significant, *F<1*, but the effect of trial was significant, *F*(8, 160)= 21.56, *p* < 0.000, *ηp*2 = .519, with time spent decreasing over trials.

*Summation BY and BZ Test trials*

More time was spent under Beacon B in the Novel quadrant (Z) than in the Inhibitory quadrant (Y) (Figure 8: Left hand Panel). Latencies to pass under Beacon B in quadrant Z were shorter than to pass under Beacon B in quadrant Y (Figure 9: Right hand Panel). These impressions were confirmed by a series of ANOVAs.

A mixed design Group (Novel v Inhibitory) x Gender (Male v Female) x Trial Type (BY v BZ) ANOVA was performed on the time spent under Beacon B during trials BY and BZ. The effect of group was not significant, *F*(1, 20) = 2.88, *p* = 0.105, *ηp*2 = .126, the effect of gender was not significant, *F*<1, but the effect of trial type was significant, *F*(1, 20)= 7.55, *p* = 0.012, *ηp*2 = .274, with more time spent under Beacon B in BZ trials than in BY trials.

A mixed design Group (Novel v Inhibitory) x Gender (Male v Female) x Trial Type (BY v BZ) ANOVA was performed on the latency to cross the platform area under Beacon B. The effect of group was not significant, *F*(1, 20) = 2.94, *p* = 0.102, *ηp*2 = .124, the effect of gender was not significant, *F*<1, but the effect of trial type was significant, *F*(1, 20)= 9.47, *p* = 0.006, *ηp*2 = .321, with shorter latencies to pass under Beacon B in BZ trials.

*Empty Pool trials*

In order to test whether the greater time spent under the beacon when it was above quadrant Z as opposed to quadrant Y was due to an unconditioned preference for quadrant Z over the other quadrants, the time spent in the pool without any beacons was measured. There was no difference in the time spent in the four platform areas (Figure 9). These impressions were confirmed by a series of ANOVAs.

A mixed design Group (Novel v Inhibitory) x Gender (Male v Female) x Platform Area (X v Y v Z v W) ANOVA was performed on the time spent in the four platform areas. The effect of group, gender and platform area were all not significant, *F*s<1, illustrating that rats did not prefer one quadrant over another.

*Retardation trials*

Numerically the escape latencies were longer for Group Inhibitory than Group Novel (Figure 10). A mixed design Group (Novel v Inhibitory) x Gender (Male v Female) x Trial ANOVA was performed on the time to reach the platform on retardation trials. Unlike in the rat study, the effect of group was not significant, *F*(1, 20) = 1.33, *p* = 0.262, *ηp*2 = .062, while the effect of gender, *F*(1, 20) = 9.86, *p* = 0.005, *ηp*2 = .330, and trial, *F*(6, 120) = 15.89, *p* < 0.000, *ηp*2 = .443, were both significant, with the latencies for males shorter than for females and latencies for both groups decreasing over trials.

General Discussion

The two key tests of CI are summation and retardation tests. In a comparison of summation test trials BY and BZ, both rat and the human participants spent less time under Beacon B and took longer to cross underneath Beacon B when it was placed in quadrant Y than when it was placed in the Novel quadrant Z. These results suggest that training an AX+ AY- discrimination led quadrant Y to become a conditioned inhibitor. The greater time spent under Beacon B in the Novel quadrant Z could not be explained by an unconditioned preference for quadrant Z. In the absence of any beacon and platforms during the Empty pool trials, rats and human participants spent no more time in quadrant Z than the other three quadrants. More importantly, the similar time spent in quadrant Y and the other quadrants in the Empty pool trial ruled out the possibility that the rats and human participants had simply learned to avoid quadrant Y. The Empty pool trial was not used in previous demonstrations of CI in a spatial domain (Sansa et al., 2009; Horne & Pearce, 2010) and thus the current findings strengthen claims that learning in the spatial domain follows the rules of associative models where stimuli compete for associative strength and Y has become inhibitory due the positive associative strength of A in the non-reinforced trials of AY- (Chamizo, 2003).

Although Horne and Pearce (2010) did not test the inhibition of Y using an Empty pool trial, they did demonstrate super normal conditioning of A following subsequent AY+ trials where the platform was present. In terms of a competitive associative model, such trials would mean that A would need to acquire further associative strength to overcome the inhibitory strength of Y. Horne and Pearce’s demonstration of super normal conditioning and the results of the empty pool trial in the current study provide strong evidence that CI was established in a spatial domain in the manner described by associative models such as that of Rescorla-Wagner (1972).

In the retardation trials, where the unmarked platform was placed in either the inhibitory quadrant (Y) or Novel quadrant (Z), escape latencies after the first trial for rats locating the platform in quadrant Y were significantly longer than those for rats locating the platform in quadrant Z. The results in the rat study could again be taken as confirmation that the Y quadrant had become a conditioned inhibitor. The results for the retardation trials were not the same for human participants. Latencies to locate the platform in the inhibitory quadrant (Y) were numerically longer than the latencies to locate the platform in the novel quadrant (Z), as in the rat study, but the difference in escape latencies did not reach significance. It is therefore difficult to conclude that Y had gained inhibitory strength. A further problem arises from the fact that the numerical difference between the groups is seen on the first trial. Despite the empty pool trials data, the human retardation data do not unequivocally reject the idea that the participants had simply learned to avoid quadrant Y in a manner suggested by Amsel and Roussel (1952), as opposed to the proposal that Y had become a conditioned inhibitor.

It may be that conditioned inhibition was not established to the same extent in the human participants in Experiment 2 as in the rats in Experiment 1. Indeed, the analysis of the behaviour during the initial BY and BX test trials revealed stronger effect sizes in the rats than in the humans, as shown by the larger partial eta values for both time in the platform area and latency to cross platform area. This reduced conditioned inhibition effect might have been due to the difference in the training procedure for the two studies. The rat study had far more trials than the human study, and the sessions were spread over several days rather than just one session. In previous spatial training experiments with humans, the current level of training was sufficient to establish spatial learning (e.g. Redhead & Hamilton, 2007). However, it may be that conditioned inhibition requires a greater number of trials over several days to establish the effect to such an extent that it would result in a difference in the retardation trials.

Comparative studies demonstrating Conditioned inhibition have been shown to provide inconsistent results (e.g. Grillon & Ameli, 2001; Artigas, Chamizo & Peris, 2001). Mitchel and Hall (2014) suggested there was little evidence of associative inhibition in demonstrations of perceptual learning in humans and that the effects were due mostly to a reduction in salience of the common cue. Without the successful retardation test in Experiment 2, it is not possible to conclude that inhibition which transferred to beacon B in the summation test was not simply due to participants avoiding Y in a manner described by Amsel and Roussel (1952).

It should be noted that there was no difference between male and female participants in Experiment 2 except in the retardation trials when males had significantly shorter escape latencies than females. Gender differences have been commonly found in virtual spatial tasks (e.g. Rosenthal, Norman, Smith, & McGregor, 2012; Allison, Redhead, & Chan, 2017), so it might be seen as surprising more differences were not found. The retardation trials were the only trials when participants had to locate a hidden platform. Thus, a difference between males and females here would be consistent with previous studies. On all other trials, the platform was marked by a beacon hanging above its location, and such marked trials might not be seen as a test of gender difference in spatial ability. What is interesting is that there was no difference between males and females in the summation trials and thus the level of inhibition was the same. This finding suggests the mechanism for CI is the same for males and females, and it also has implications for addressing the gender difference in spatial tasks.

For the Y quadrant to become inhibitory, participants must be able to discriminate between AX trials and AY trials based on the distal cues around the pool. The summation trials suggest females were able to discriminate the various quadrants of the pool as well as the males. Alison et al. (2017) suggested that greater spatial anxiety might be responsible for the poor performance of the females in their study. In the current study, by marking the position of the platform, any anxiety regarding the spatial task of locating the platform might have been reduced, allowing the females to perform the spatial discrimination as well as the males.

Overall, these results support other demonstrations that spatial learning in rats (Redhead, Roberts, Good, & Pearce, 1997) follows rules that are predicted by associative models. The results of the rat study support and extend the previous findings of conditioned inhibition in the spatial domain (Horne & Pearce, 2010; Sansa et al., 2009). Previous research has shown that phenomena such as Overshadowing and Blocking have been demonstrated in human spatial learning experiments (Redhead & Hamilton, 2007; Redhead, Hamilton, Parker, Chang, & Allison, 2013). The current study with human participants is the first showing Conditioned Inhibition within a human spatial task, although due to the non-significant retardation test it is not possible to conclude the observed CI was governed by associative models. Finally, given the extensive use of virtual watermazes in human designs to measure spatial impairment in clinical populations (e.g. Barkas, Taylor, Hamilton, Redhead, & Gray, 2010; Barkas et al., 2012), the similarities between the results of the rat study and the human study in the current paper support the validity of the virtual watermaze as a tool to measure spatial learning if not the mechanisms by which associations and inhibition are acquired.

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Figure Captions

*Figure 1*. Pool layout, and platform and beacon positions during training, summation, empty pool and retardation trials.

*Figure 2*. Mean Escape Latencies and time under Beacon A during training for rats. Latency to locate platform under Beacon A in quadrants X and W (filled circles); latency to locate platform under Beacon B in quadrants X and W (filled triangles); time spent in platform area under Beacon A in Y quadrant (empty circles). The error bars are the standard error of the mean.

*Figure 3*. Left Hand Panel Mean time spent under the Beacon B during the Summation trials BY (black column) and BZ (grey column) for rats. The standard error bars are the standard error of the mean. Right Hand Panel: Mean latency to cross underneath Beacon B during the Summation trials BY (black column) and BZ (grey column). The error bars are the standard error of the mean.

*Figure 4*. Mean time spent in the platform areas of quadrants W (light grey column), X (dark gray column), Y (black column) and Z (white column) during the Empty pool trials for Non-Human Animals. The error bars are the standard error of the mean.

*Figure 5*. Mean group escape latencies during retardation trials. Latency for rats in Group Inhibit to locate platform in quadrant Y (empty circles); latency for rats in Group Novel to locate platform in quadrant Z (filled circles). The error bars are the standard error of the mean.

*Figure 6*. Left hand Panel: Plan of pool and room. Right hand Panel: View across pool from SW quadrant

*Figure 7*. Mean Escape Latencies and time under Beacon A during training for human participants. Latency to locate platform under Beacon A in quadrants X and W (filled circles); latency to locate platform under Beacon B in quadrants X and W (filled triangles); time spent in platform area under Beacon A in Y quadrant (empty circles). The error bars are the standard error of the mean.

*Figure 8*. Left Hand Panel Mean time spent under the Beacon B during the summation trials BY (black column) and BZ (grey column) for human participants. The error bars are the standard error of the mean. Right Hand Panel Mean latency to cross underneath Beacon B during the summation trials BY (black column) and BZ (grey column) for human participants. The error bars are the standard error of the mean.

*Figure 9*. Mean time spent in the platform areas of quadrants W (light grey column), X (dark grey column), Y (black column) and Z (white column) during the Empty pool trials for human participants. The error bars are the standard error of the mean.

*Figure 10*. Mean group escape latencies during retardation trials for human participants. Latency for participants in Group Inhibit to locate platform in quadrant Y (empty circles); latency for participants in Group Novel to locate platform in quadrant Z (filled circles). The error bars are the standard error of the mean.

AX+

Z

W

Y

X

Y

X

Figure 1

Z

X

Platform

Platform area

Beacon A

W

Y

Beacon B

Z

Training Trials

AY-

AX+

BX+

W

Y

W

Z

X

Z

X

AW+

BW+

W

W

Y

Y

Summation test trials

Z

X

Z

X

BZ-

BY-

W

Y

W

Y

Empty pool test trials

W

Z

X

Y

Y+

Z

W

X

Z+

Y

Retardation trials

Z

X

Figure 2



Figure 3

Figure 4



Figure 5



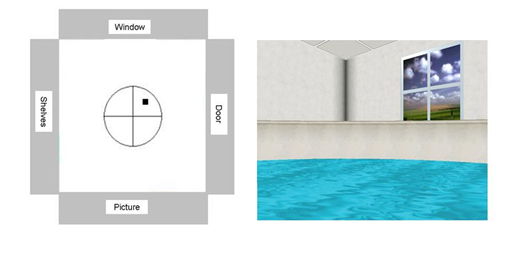


Figure 6

Figure 7

Figure 8



Figure 9



Figure 10

**

Highlights

Demonstration of Conditioned inhibition in spatial domain in both Rats and Human studies

Results suggest Spatial learning in both humans and rats follow rules governed by associative models

Similarity of results in the studies add validity to use of digital watermaze to measure spatial learning