



### Establishing a platform mediated avoidance paradigm in female rats

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Behavioural responses to learned threats can be complex, decision based and hierarchical in nature, such as the halting of foraging for food or the active avoidance of cues associated with danger. Avoidance is a natural and adaptive response that allows animals and humans to modulate their exposure to threats. Avoidance can easily become maladaptive and is used as a common diagnostic tool for anxiety, phobias, obsessive compulsive disorders and depression- all of which have a far higher incidence in females. Therefore, the development of animal models of avoidance specifically female models, may give rise to new approaches to treatment and understanding of disorders underpinned by excessive avoidance. We have applied an active avoidance task - Platform Mediated Avoidance (PMA), in which female Sprague Dawley rats learnt to avoid a tone-signalled footshock by stepping onto a non-conductive platform. The PMA paradigm consisted of two habituation days, in which animals were exposed to the conditioning chamber in the absence of tone or shock and were presented with novel food sources to encourage naturalistic exploration. During the 10 subsequent days, animals were returned to the same conditioning chamber and periodically exposed to 30 second tones (30 s, ~4 kHz, ~75 dB) that co-terminated with a mild 2 second footshock (0.4mA). Over the 10 days, female rats were exposed to 9 tone-shock presentations per day. Testing day occurred after the 10 training days, and consisted of three tone presentations over 10 minutes in the absence of shock. Successful avoidance was defined by an animal having all 4 paws on the platform and was measured at three time points: the 300 seconds pre-tone, during 30s tone, and in the final 2 seconds of tone which would usually be indicative of a shock. At test, shock rats spent ~2x more time on the platform pre-tone compared to control rats (n=11, shock= 194.1s ± 56.68; n=8, control= 65.92 ± 70.54 , t=4.395, p=0.0004), and spent ~3x more time on the platform during 30s tone presentations (n=11, shock= 26.98s ± 5.44; n=8, control= 7.11s ± 12.37, t=4.667, p=0.0002). During the last 2 seconds of tone on test day, 90% of shock females exhibited successful avoidance, indicating that Shock females successfully learnt the association between tone and footshock and that the footshock could be avoided by moving onto the platform. Two hours after final tone presentation on test day, all rats were deeply anaesthetised (isoflurane followed by i.p. pentobarbitone) and were transcardinally perfused with 4% formaldehyde. Brains were then sectioned and immunolabelled for Fos-protein, a marker for neuronal activation, in order to eventually explore regional activation during PMA. In future experiments, we would like to explore the role Oxytocin, a neuropeptide, may play as an anxiolytic in PMA, with the potential to translate these findings to human mental health conditions underpinned by excessive avoidance.