Supplemental Information

Methods

Since sex differences have been observed in multiple motivational tasks (Van Haaren *et al.*, 1990; Jonasson, 2005; Becker, 2009; Dalla & Shors, 2009; Sutcliffe, 2011) and should be examined in new animal models (Becker *et al.*, 2005; Beery & Zucker, 2011), we tested the effects of reward reduction and omission on choice preference in both male and female rats.

Vaginal lavage was performed routinely on female rats during the dark phase to track estrous cycle stage (Becker et al., 2005). An eyedropper gently inserted into the vaginal canal injected and withdrew approximately 0.3 ml saline. The eyedropper was rinsed with distilled water before each lavage to prevent cross-contamination among samples. The vaginal cells in the saline solution were viewed under a light microscope and documented by standards previously described (Tropp & Markus, 2001; Becker *et al.*, 2005). Specifically, smears containing predominately cornified cells were classified as estrus. Smears containing a mixture of cornified and leukocytes were classified as metestrus. Smears containing primarily leukocytes were classified as diestrus, and smears containing predominantly nucleated epithelial cells were classified as proestrus. Consistent with previous experiments (Tropp & Markus, 2001), the relatively mild level of food restriction did not prevent the female rats from cycling. Female and male rats were trained identically and given the negative contingency switch once they exhibited stable behavioral responding on the operant task.

Results

No sex differences were found in the behavioral task.

Both male and female rats earned rewards from both levers, showing no reliable preference for one lever over the other during free choice trials, especially given that each lever was equally rewarded (Supplemental Fig. 1A; main effect, $F_{(1,23)} = 1.389$, $p = 0.251$; males, $p = 0.819$; females, $p = 0.143$). No significant sex difference was observed in baseline performance on free choice trials (Supplemental Fig. 1A; F(1,23) = 0.778, p = 0.387).

Both male and female subjects learned to distinguish between the two cue lights with near perfect accuracy revealing no side bias during forced choice trials (Supplemental Fig. 1B; main effect, F(1,23) = 0.801, p = 0.380; males, p = 0.328; females, p = 0.827), and performance between male and female rats did not significantly differ on forced choice trials (Supplemental Fig. 1B; F(1,23) = 2.375, p = 0.137). Furthermore, male and female rats did not significantly differ in number of sessions to meet criterion for stable responding (FR4: males mean 3.73 ± 2.41, females means: 5.00 ± 2.65, t(22) = -1.222, p = 0.235).

Reducing reward by 50% on one lever did not initially induce a behavioral preference (main effect $F_{(1,15)} = 0.178$, $p = 0.679$). Specifically, during the first session of reward reduction neither male ($p = 1.000$) nor female ($p = 0.448$) subjects exhibited a behavioral preference for one lever over the other during free choice trials (Supplemental Fig. 2A), and there was no difference in choice behavior between males and females ($p = 0.679$). By the second ($F_{(1,15)} = 43.986$, $p < 0.001$) and third ($F_{(1,15)} = 39.656$, $p < 0.001$) sessions of reward reduction, male (Supplemental Fig. 2B; session 2, $p = 0.005$; session 3, $p = 0.006$) and female (Supplemental Fig. 2C; session 2, $p < 0.001$; session 3, $p < 0.001$) subjects showed a significant preference for the lever yielding twice as much reward. These data demonstrate that male and female rats learned this contingency switch; however, a 50% reduction in reward was not a salient enough reduction to prompt an immediate alteration in behavior.

In contrast to reward reduction, when the reward following a correct operant response on one lever was unexpectedly omitted (i.e. reduced to 0 pellets), both male ($p < 0.001$) and female ($p < 0.001$) subjects displayed a robust behavioral preference for the rewarded lever during the very first session (Supplemental Fig. 2D; main effect $F_{(1,17)} = 55.006$, $p < 0.001$) with no sex differences ($p = 0.513$). A strong preference for the rewarded lever continued during free choice trials of the second session ($F_{(1,17)} = 475.959$, $p < 0.001$) and third session ($F_{(1,17)} = 2{,}517.712$, $p < 0.001$) of reward omission in males (Fig. 2E; $p < 0.001$) and females (Fig. 2F; $p < 0.001$).

The lack of sex difference in development of choice preference was not due to estrous cycle effects in female subjects.

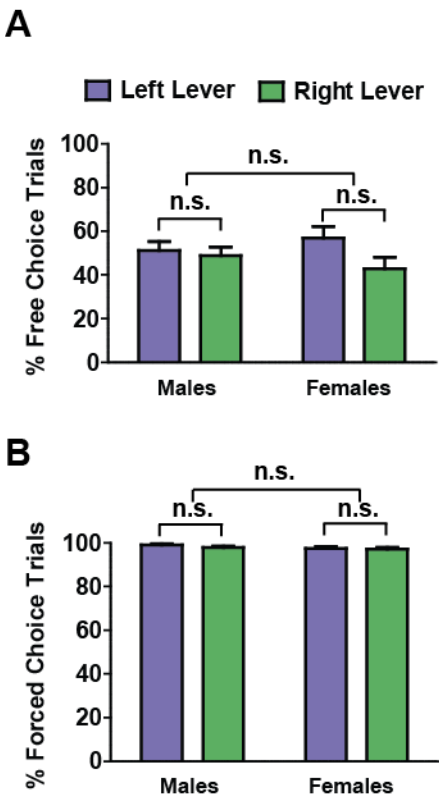
Estrous cycle stage for each female rat was determined via vaginal lavage (Becker et al., 2005) for all reward reduction and omission sessions. Samples containing primarily leukocytes were classified as diestrus (Supplemental Fig. 3A). Smears containing predominantly nucleated epithelial cells were classified as proestrus (Supplemental Fig. 3B). Smears containing predominately cornified cells were classified as estrus (Supplemental Fig. 3C). Samples containing a mixture of cornified and leukocytes were classified as metestrus. Metestrus and diestrus data were combined for analysis (Lynch et al., 2000), and estrous cycle stage was included as a covariate in analysis (Girard & Garland Jr, 2002; Pawluski *et al.*, 2006) to determine if it modulated the development of the behavioral preference.

Female subjects in all stages of the estrous cycle performed similarly to males (Supplemental Fig. 3D-I). Indeed, estrous cycle did not affect development of choice preference during the first (Supplemental Fig. 3D; $F_{(1,14)} = 0.001$, $p = 0.973$), second (Supplemental Fig. 3E; $F_{(1,14)} = 1.327$, $p = 0.269$) or third (Supplemental Fig. 3F; $F_{(1,14)} = 0.015$, $p = 0.905$) session of reward reduction. Similarly, estrous cycle stage did not affect behavioral preference in the first (Supplemental Fig. 3G; $F_{(1,16)} = 0.669$, $p = 0.425$), second (Supplemental Fig. 3H; $F_{(1,116)} = 2.135$, $p = 0.163$), or third (Supplemental Fig. 3I; $F_{(1,16)} = 0.002$, $p = 0.970$) session of reward omission.

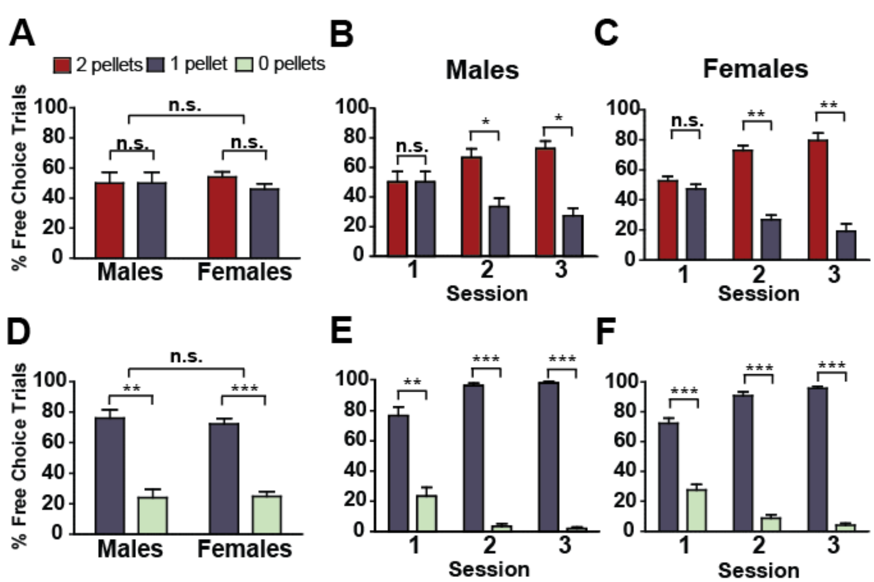
In conclusion, female rats showed similar behavior as male rats in both reward reduction and omission tests, and estrous cycle stage did not affect choice preference during either contingency switch. These results are consistent with foraging studies in the field (Clark, 1980). Similarly, other laboratory studies have neither found sex differences during certain operant tasks (Van Haaren *et al.*, 1987; Van Hest *et al.*, 1988; 1989; Stratmann & Craft, 1997; Carroll *et al.*, 2009) nor estrous cycle effects on operant performance (Stratmann & Craft, 1997; Davis *et al.*, 2008; Cummings *et al.*, 2011). Since male and female rats performed similarly on this behavioral task (Supplemental Fig. 1) and no sex differences were observed in latency to develop a behavioral preference in either of the negative contingency switches (Supplemental Figs. 2&3), male subjects were used to elucidate the roles of D1- and D2-like receptors in developing a choice preference following unexpected reward omission. While the possibility exists that the neuronal mechanisms may differ between males and females (De Vries, 2004), male and female rats have both been used to study fundamental motivational systems, including the nucleus accumbens' control of appetitive and aversive behaviors (Reynolds & Berridge, 2001; 2008), not revealing significant sex differences when conditions are related to fundamental aspects of foraging.

Supplemental Figures

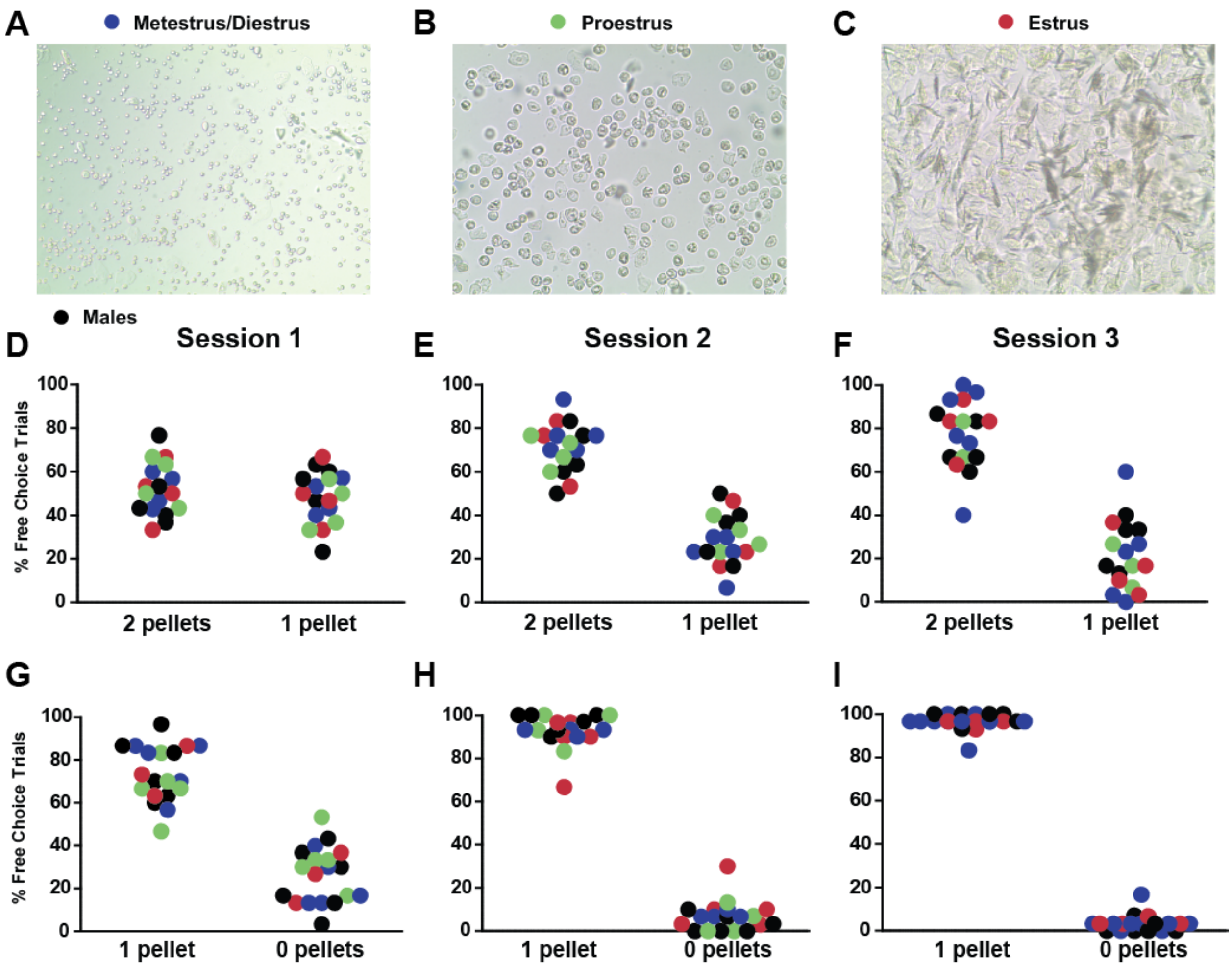
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure Legends

Supplemental Figure 1: Male and female rats performed the task equally well ***A,*** Neither male nor female rats exhibited a significant preference for one lever or the other. ***B,*** Both males and females completed forced choice trials with near perfect accuracy. n.s. = not statistically significant. Error bars indicate mean ± SEM.

Supplemental Figure 2: Male and female rats exhibited the same choice preferences during the two negative contingency switches. ***A-C,*** Neither male nor female rats displayed a choice preference during the first session of reward reduction (**A**). Both male (**B**) and female (**C**) rats displayed a preference for the lever yielding greater reward during the second and third sessions of reward reduction. ***D-E***, Both males and females exhibited a significant preference for the rewarded lever during the first reward omission session (**D**). Males (**E**) and females (**F**) similarly showed a robust preference for the rewarded option during all sessions of reward omission. n.s. = not statistically significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Error bars indicate mean ± SEM.

Supplemental Figure 3. Estrous cycle did not affect the development of choice preference. ***A-C,*** Representative images of vaginal epithelial cells in diestrus (**A**), proestrus (**B**), and estrus (**C**). ***D-F,*** Female rats in each estrous cycle stage performed similarly to males (black circles) on the first (**D**), second (**E**), and third (**F**) session of reward reduction. ***G-I,*** During the first (**G**), second (**H**), and third (**I**) session of reward omission, females in each estrous cycle stage performed similarly to males.

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