

### Category E Explanation

1. Registration Number: 14-R-0036
2. Number: 27 (22 of these 27 animals also received acclimation and neuroimaging as described below)
3. Species: marmoset

#### **Behavioral Testing: Temporary Separation from Colony**

The marmoset is removed from its home cage and placed in a different room in a novel cage for 7 hours with no visual or auditory access to conspecifics. Urine cortisol levels are measured before (day prior), after (day after), and hourly during the isolation to monitor changes in cortisol levels from baseline to post-separation. The environmental enrichment is similar to what the animals normally receive. This procedure is only administered once during the year.

Since the goal of the study is to determine sex differences in emotional reactivity to a stressor, there is no alternative to our temporary isolation procedure, a mild stressor that only elicits transient responses in marmosets. During these procedures, we cannot deliver any anesthetic, analgesic, or tranquilizing drugs to alleviate any potential mild stress associated with the procedure. These drugs would mask the behavioral responses being measured as well as change the levels of cortisol within the urine samples being collected.

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### Category E Explanation

1. Registration Number: 14-R-0036
2. Number: 22 (22 of the same 27 animals listed above for temporary separation)
3. Species: marmoset

#### **Marmoset acclimation and neuroimaging procedures**

Neuroimaging techniques allow recording of brain activity in humans and animals in a noninvasive manner. For awake neuroimaging, the animals need to be restrained, as head movements prevent the successful acquisition of brain images. In addition, the scanner generates noise. Both the restraint and the noise are somewhat stressful, which is why we use acclimation procedures prior to imaging to minimize the stress for the animal.

For this procedure, we follow state-of-the-art methods developed at NIH, which ensure that the animal is fully habituated before actual neuroimaging and which emphasize the animal's comfort in the restraint apparatus. Prior to neuroimaging, animals undergo at least 5 mock neuroimaging sessions (i.e., acclimation), in which the animal is progressively habituated to the restraint apparatus and the noise of the scanner. Only animals who are fully habituated at the end of the mock sessions move on to neuroimaging. During neuroimaging, animals are scanned awake so that we can image their brain activity. We cannot deliver any anesthetic, analgesic, or tranquilizing drugs, as these drugs would interfere with the patterns of brain activity and would therefore adversely affect the data collected.

We have not experienced any adverse effects with the neuroimaging procedure. Animals are carefully monitored for any signs of lethargy, failure to eat, and/or diarrhea. If any of these symptoms were to occur, the procedures would be stopped, a veterinarian would immediately be consulted, and appropriate treatment would be given. We monitor the animal's health daily, including behavioral observations and weekly weight checks.