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Visual sensation seeking with caloric-restriction and amphetamine administration in rats: A model for investigating mood and approach motivation --Manuscript Draft--

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Abstract:	<p>Mood state affects perception, thoughts, and approach behavior. Although the investigation for understanding neural mechanisms of mood state and approach motivation in animal models has long been underway, such investigations typically depend on extensive conditioning training. We recently developed a model system that requires little training and thereby can be applied more easily than conditioning-based models for the investigation of these mechanisms. Brief presentation of light stimuli (or visual sensation, VS) has long been known to reinforce approach responses in rats. Rats typically learn within a single session to display VS seeking responding, and maintain similar response levels over many repeated sessions. Thus, it is easy to execute and maintain VS seeking over many sessions. The vigor of VS seeking markedly increases in rats maintained on chronic caloric restriction (CR) and receiving the administration of amphetamine (AMPH). Rats receive limited amounts of food daily for two weeks or longer in such a way that 85-90% of their original weights are maintained. Immediately after an intraperitoneal injection of amphetamine (1 mg/kg), rats are individually placed in test chambers for VS seeking behavior. The described procedure is easy to execute and produces reliable enhancement in approach responding, and thus, appears to provide a useful model system to address neurobiological mechanisms involved in motivation and mood. Furthermore, the model may be useful in research on synergistic interaction between CR and psychoactive drugs and on psychiatric illnesses including mood, obsessive-compulsive, and attention deficit hyperactivity disorders.</p>
Author Comments:	Dear Editorial Staff, We described a procedure involving so-called sensation seeking behavior that is maintained by flash of light. Rats and other laboratory animals have been known to seek light stimuli given opportunities. We recently found that this visual-sensation

	<p>seeking behavior is augmented synergistically by the combination of chronic food-restriction and amphetamine administration. One or the other manipulation alone does not have nearly as much effect. This phenomenon should be fully known among researchers and clinicians, because this finding has potentially important clinical implications. We thought that the publication of the procedure in JoVE would help them appreciate this phenomenon easily.</p> <p>Satoshi Ikemoto</p>
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TITLE: Visual sensation seeking with caloric-restriction and amphetamine administration in rats:
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Amphetamine; Dietary restriction

SHORT ABSTRACT:

The biomedical research community will benefit from having an animal model that is easy to execute and maintain for investigating neural mechanisms regulating motivation and mood. Here we describe a model system involving operant responding reinforced by light illumination with caloric restriction and amphetamine administration in rats.

LONG ABSTRACT:

The investigation for understanding neural mechanisms of mood state and approach motivation in animal models has long been underway. Such investigations typically depend on extensive conditioning training. We recently developed a model system that requires little training and thereby can be applied more easily than conditioning-based models for the investigation of these mechanisms. Brief presentation of light stimuli (or visual sensation, VS) is known to reinforce approach responses in rats. Rats typically learn within a single session to display VS seeking responding, and maintain similar response levels over many repeated sessions. Thus,

it is easy to execute and maintain VS seeking over many sessions. The vigor of VS seeking markedly increases in rats maintained on chronic caloric restriction (CR) and receiving the administration of amphetamine (AMPH). Rats receive limited amounts of food daily for two weeks or longer in such a way that 85-90% of their original weights are maintained. Immediately after an intraperitoneal injection of amphetamine (1 mg/kg), rats are individually placed in test chambers for VS seeking behavior. The described procedure is easy to execute and produces reliable enhancement in approach responding, and thus, appears to provide a useful model system to address neurobiological mechanisms involved in motivation and mood. Furthermore, the model may be useful in research on synergistic interaction between CR and psychostimulant drugs and on psychiatric illnesses including mood, obsessive-compulsive, and attention deficit hyperactivity disorders.

INTRODUCTION:

Mood state affects perception, thoughts, and approach behavior. Approach behavior is broadly defined as many types of responses that would or might lead to life-sustaining or promoting stimuli or events (e.g., food and copulation; i.e., positive reinforcers), including reward-reinforced and exploratory responses¹. Drugs of abuse may not help to sustain or promote life, but reinforce behavioral responses, a property that is thought to arise from their capacity to stimulate approach motivation and mood process²⁻⁴. The scientific pursuit to understand the neural mechanisms that regulate mood and approach motivation has long been underway^{e.g., 1,5-8}.

Such investigations typically depend on extensive conditioning training, because it is necessary to examine approach behavior in the absence of primary reinforcers like food and drugs. This is important because mechanisms for approach behavior differ from those for consummatory processes of primary reinforcers^{1,9}. In this regard, exploratory behavior may be useful for studying approach motivation mechanisms, since it does not require training. However, it is often difficult to determine whether behavioral responses indicate exploration, mere locomotion or something else. We recently developed a model system for mood and approach motivation, which overcomes these issues, and can be thereby applied more easily than conditioning- or exploration-based models for the investigation of their mechanisms¹⁰. The purpose of the present article is to describe the procedure of this model system in detail.

Brief presentation of light stimuli, or visual sensation (VS), has long been known to reinforce approach responding in rats and other species^{11,12}. Rats typically learn within a single one-hour session to prefer pressing on the lever that produces a one-second light illumination over the lever that has no programmed consequence (Fig. 1 and 2)^{10,13}. Moreover, they maintain similar response levels over many repeated sessions. Thus, it is easy to execute and maintain responding reinforced by VS. However, the level of VS seeking remains relatively low when no other manipulation is employed. This is a problematic when experimental manipulations to examine underlying mechanisms are expected to decrease VS seeking responding, since there is little room to detect varying manipulation effects. We found that the vigor of VS seeking markedly increases in rats maintained on chronic caloric restriction (CR) that have received an

administration of amphetamine (AMPH) just prior to testing ¹⁰. We propose VS seeking combined with CR and AMPH administration as a model system for the investigation of neural mechanisms involved in mood and approach motivation.

PROTOCOL:

The following behavioral procedures are in accordance with protocols approved by the Animal Care and Use Committee of the National Institute on Drug Abuse Intramural Research Program and the *Guide for the care and use of laboratory animals* ¹⁴.

1. Animals

1.1. Obtain male Wistar rats from an accredited farm, and initially house them in pairs in a vivarium on a 12-hr reverse light dark cycle (lights on 7am and lights off 7pm) with free access to food and water.

1.1.1 House rats in pairs so they can benefit from social interactions while maturing.

1.1.2 Keep rats on a reversed light schedule, which makes it easier to test them during their active (i.e., dark light-cycle) phase. It is unclear whether this schedule makes a significant difference in performance.

2. Caloric Restriction and Monitoring Body Weight

2.1. Start the CR procedure when rats weigh between 350-400g (12-15 weeks old).

Note: Rats weighing less than 350g may be too immature to start CR, and rats weighing more than 450g may not effectively respond to CR for subsequent VS seeking tests, although this has not yet been systematically evaluated.

2.2. Weigh rats and record their weights. This is their original or starting weight.

2.3. Remove all food from the home cage, but leave water. At this point, house animals individually to properly control their food intake. Note: The procedure could be used in combination with brain manipulations that require cranial probes, in which case animals should be housed individually from the time of cranial implantation to avoid housing partners tampering with implants.

2.3.1 If research design requires an *ad libitum* control group, set aside a subset of the animals for AL feeding and do not remove their food. Other than their diet, treat this control group the same as the CR group.

2.3.2 Assign rats to groups in such a way that groups do not differ with respect to their original body weight unless group size is large (>20).

2.4. Weigh rats daily and at the same time of day throughout the experiment.

Note: Time of feeding in relation to testing does not appear to significantly affect VS seeking responding. Rats that are fed just prior to testing perform as well as rats that are fed after testing (A. Talishinsky and S. Ikemoto, unpublished observation).

2.5. Give limited amounts of standard rodent chow to rats once a day.

2.5.1 Start with providing 10g on the first day or two and then adjusting the amount (6-15g based on how the animal's body weight has changed from the day before).

2.5.2 Gradually bring weights down to 85%-90% of their initial (pre-food restriction) weights over the period of about one or two weeks, and maintain them at that weight for the remainder of the protocol.

2.6. During the CR period prior to testing, make sure that rats are well adjusted to daily handling as they are being weighed. This is important since anxious rats may not display approach responding during testing.

2.6.1 If rats are severely anxious, give them extra time to get habituated to the experimenter's handling. However, do this without biasing one group relative to the other.

2.7. Maintain CR-induced reduction in body weight for at least 2 weeks before behavioral testing. CR lasting less than a week does not significantly increase VS-seeking when paired with AMPH ¹⁰.

3. Operant Conditioning Chamber and Visual Sensation Programing

3.1. Prepare operant chambers.

3.1.1 Equip each operant conditioning chamber (Figure 1; 30 x 22 x 24 cm) with two levers (45 mm wide and 62 mm thick, protruding 20 mm from the wall), two cue lamps (100-mA incandescent light bulbs) with white covers, a house lamp (100-mA bulb painted red), four pairs of infrared detectors (separated by 6 cm), and a small video camera to monitor and record the animal's behavior. Control the hardware by computer software and programs.

3.2 Place the chamber in a box that seals it from external stimuli including noise and lights so that rats are not distracted by unintended stimuli.

3.3. Program the operant conditioning chamber in such a way that a depression on one lever (active lever) illuminates the cue light above the lever for 1 sec and turns off the house light for 5 sec. During the 5-sec house-light-off period, all lever pressing is counted, but produces no programmed consequences. Pressing on the other lever (inactive lever) has no programmed consequences throughout the session.

3.3.1. Consider using a progressive ratio reinforcement schedule in which the number of active leverpresses required to produce a presentation of VS increased by 1 every 10 VS presentations throughout the experiment, to increase active lever presses over inactive presses. Note: Other reinforcement schedules such as a variable-ratio 2 schedule can be employed. See discussion.

3.4. Counterbalance the assignment of left and right levers for active and inactive levers, so that the difference in pressing between the two levers can be attributed to VS reinforcement, and not to the location.

3.5. Arrange the infrared detectors, and program them in such a way that infrared beam between the cells is interrupted as rats move about the chamber. Use these counts to quantify locomotor activity (crossing).

Note: instead of infrared beams, video camera-assisted tracking systems can be used to monitor locomotor activity. Regardless of the procedure, it is important to monitor locomotor activity (see discussion).

3.6. Set program to record the following data: active and inactive lever presses, VS earned, and locomotor activity (crosses).

4. Visual-Sensation Seeking Behavior

4.1. To habituate animals to the testing chamber and to gather baseline VS seeking data, place rats individually in the operant conditioning chambers for two days before test day and run the VS seeking program.

4.2. Habituate rats with intraperitoneal (IP) injections prior to the test by injecting saline (1mL/kg) 1 or 2 times at least one day before the test session.

4.2.1 Perform IP injections without stressing rats, and ensure that the experimenter who gives the injections is consistent (same person every time). Stress like fear/anxiety may disrupt leverpress performance.

4.3. On the test day, inject each rat with AMPH. Immediately after injections, place rats individually in the operant conditioning chambers and run the behavioral testing for 60 min.

4.3.1 Use the 1-mg/kg dose of AMPH, which is most effective in augmenting VS seeking behavior (Figure 3). A 2.5-mg/kg dose of methylphenidate is similarly effective (A. Talishinsky and S. Ikemoto, unpublished observation).

REPRESENTATIVE RESULTS:

Rats acquire VS-seeking behavior without any shaping procedure, although the level of VS

seeking is moderate. Figure 2 shows how rats display VS seeking over a 2-week course of repeated tests in chronic CR and *ad libitum* (AL)-fed rats. The weights of these groups gradually deviated over 12 days (Figure 2d). Rats respond on the active lever more than inactive lever (Figure 2a, b), and hence the presentation of VS is moderately reinforcing. CR alone tends to slightly increase VS seeking, while having no apparent effect on locomotor activity (Figure 2c).

AMPH augments VS seeking in CR rats much more effectively than in AL rats (Figure 3). Injections of AMPH dose-dependently alter VS seeking. The 1-mg/kg dose of AMPH powerfully augments VS-reinforced lever pressing in CR rats. The 3-mg/kg dose actually attenuates VS seeking, decreasing active lever presses similar to the level of saline, while the dose is still effective in increasing locomotor activity. Notice also that while AMPH doses have similar effects on locomotor activity between CR and AL groups, they have distinct effects on VS-reinforced lever presses between the groups.

Figure Legends:

Figure 1

Operant conditioning chamber. Displaying location and orientation of the two levers (45 mm wide and 62 mm thick, protruding 20 mm from the wall), two cue lamps with white covers, house lamp with a red bulb, and four pairs of infrared detectors (separated by 6 cm).

Figure 2

Effects of caloric restriction (CR) on VS seeking, locomotor activity and weights. Data are means \pm SEM. Effects of CR on active lever presses (a), inactive lever presses (b), locomotor activity (c) and body weights (d). * $p < 0.05$, food-restriction value being significantly different from its session-1 value. + $p < 0.05$, AL value being significantly different from its session-1 value. The data are adopted from Keller *et al.* (2014) with permission from Springer.

Figure 3

Effects of AMPH on VS-reinforced responding. Data are means \pm SEM. * $p < 0.05$, ** $p < 0.01$, food-restriction value being significantly different from its 0-mg/kg value. + $p < 0.05$, AL value being significantly different from its 0-mg/kg value. # $p < 0.05$, dose value (AL and CR groups combined) being significantly different from its 0-mg/kg value. @ $p < 0.005$, CR value being significantly different from corresponding AL value. The data are adopted from Keller *et al.* (2014) with permission from Springer.

DISCUSSION:

The present article has described a behavioral procedure that provides a useful model system for investigations of neurobiological underpinnings of mood and approach motivation by using a VS-seeking procedure with the synergistic action of CR and AMPH. Particular advantages of this procedure over conditioning-based procedures are that training is minimal, and rats can be tested repeatedly over multiple sessions. In addition, compared to exploratory behavior-based

procedures, it is easier to interpret whether rats are engaging in approach behavior with the VS-seeking procedure. Indeed, although locomotor activity may be used as a measure of exploratory behavior under certain conditions, we found that amphetamine administration has differential effects on VS responding and locomotor activity between CR and AL states (Figure 3)¹⁰.

The most important step in succeeding with the present procedure is to have rats that are comfortable with experimenters and test chambers. Rats are naturally curious and voluntarily explore their environment, an activity that is essential for rats to discover that leverpressing results in VS presentations. Anxiety suppresses approach behavior¹⁵, including exploration, and thereby decreases VS seeking. AMPH could be substituted with methylphenidate, since we recently found similar synergistic effects of CR and methylphenidate on VS seeking (A. Talishinsky and S. Ikemoto, unpublished observation). It is also possible to use different light stimuli¹⁶⁻¹⁸, although it is unclear at this time how the intensity and duration of light influence VS seeking. Different reinforcement schedules have also been employed effectively. We have used a variable ratio 2 schedule in which VS is presented following 1, 2, or 3 responses on the active lever, and the required number changed randomly from trial to trial. We obtained similar levels of responding between the progressive ratio and variable ratio schedules¹⁰.

The VS-seeking procedure sensitized with CR and AMPH is intended for experiments where manipulations are expected to disrupt VS-seeking. However, when the possible outcome of manipulations is to increase VS-seeking responding, the VS-seeking procedure may be executed without CR or AMPH. Indeed, previous studies successfully determined that approach motivation is potentiated by the stimulation of the mesolimbic dopamine system or by the inhibition of median raphe neurons, using the VS-seeking procedures without the use of CR^{1,13,19,20}.

The use of VS with CR and AMPH has an obvious limitation. It is not clear to what extent the mechanisms found with this procedure are generalized to those of mood and general approach motivation. Therefore, the data should be interpreted with care. However, this issue can be dealt with by conducting follow-up experiments involving approach behavior maintained by sensory stimuli conditioned with other reinforcers, after potentially important findings with VS with CR and AMPH are made.

The present model may also contribute to clinical issues. The potentiated VS-seeking model in rats may be useful as a model system for attention deficit hyperactivity disorder (ADHD) to investigate how psychostimulant drugs reverse inability to focus, or for mania as in bipolar disorder or obsessive-compulsive disorder (OCD) to investigate what mechanisms are involved in pathophysiology of compulsive behavior.

In addition, it is potentially important to investigate how CR and psychoactive drugs synergistically increase VS seeking. Although it has not yet been documented whether the administration of psychoactive drugs can indeed interact with CR in human populations, it is tempting to predict, on the basis of the Keller et al. (2014) finding¹⁰, that some psychoactive

drugs like AMPH or lithium will augment approach behavior in calorie-restricted humans. Psychostimulants such as AMPH or methylphenidate are extensively used in the clinical setting because of their properties to improve focus and to decrease appetite and fatigue ²¹⁻²³, and are abused to elevate mood and produce euphoria ²⁴⁻²⁷. It is especially concerning when psychostimulants are used on a regular basis, which can cause weight loss ^{21,28}. Chronic users who have experienced weight loss may not only respond to salient environmental stimuli in an intense manner, but may also intensify drug seeking and taking, as found in rats ²⁹⁻³¹. Therefore, CR could provide positive feedback to exacerbate the use of psychoactive drugs. It is important to first document if such is the case in human populations, and then to investigate their mechanisms.

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DISCLOSURES:

The authors have nothing to disclose.

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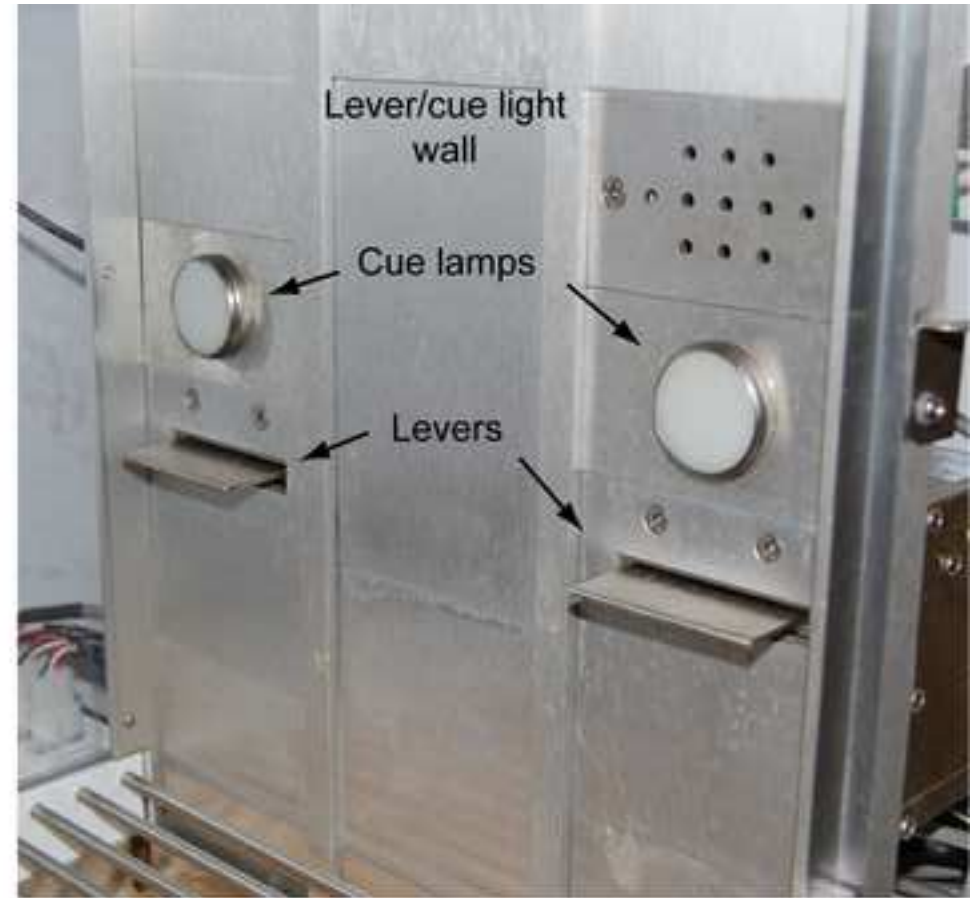
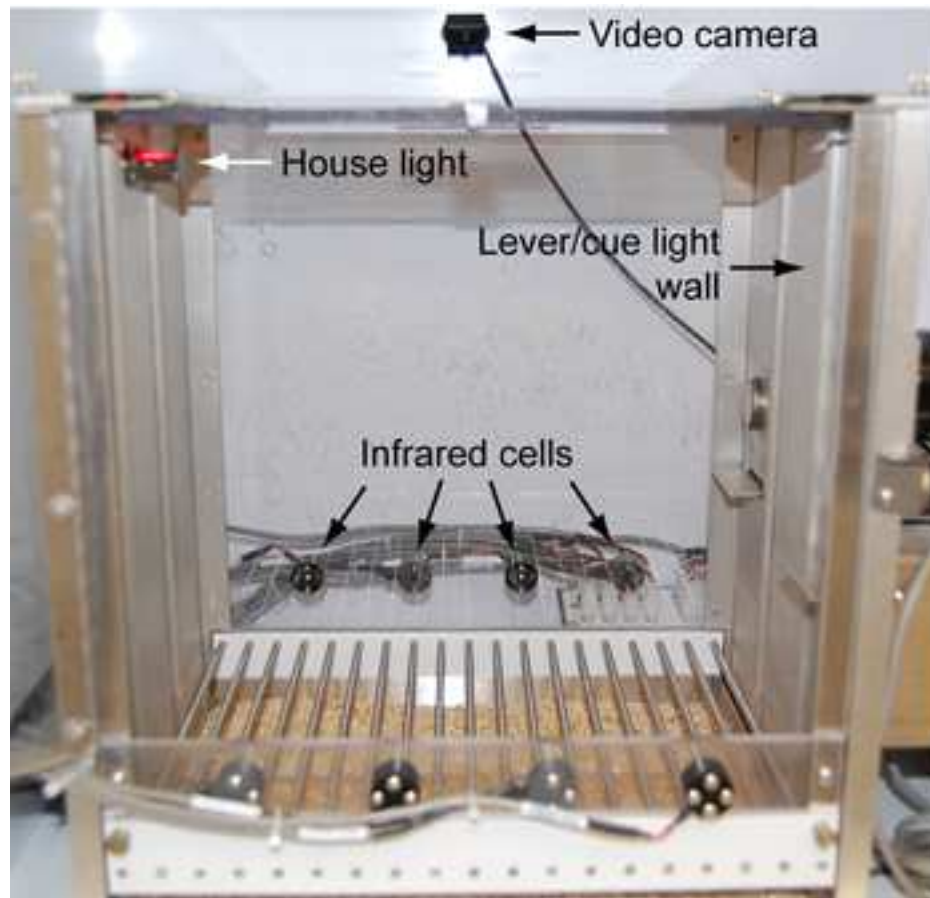
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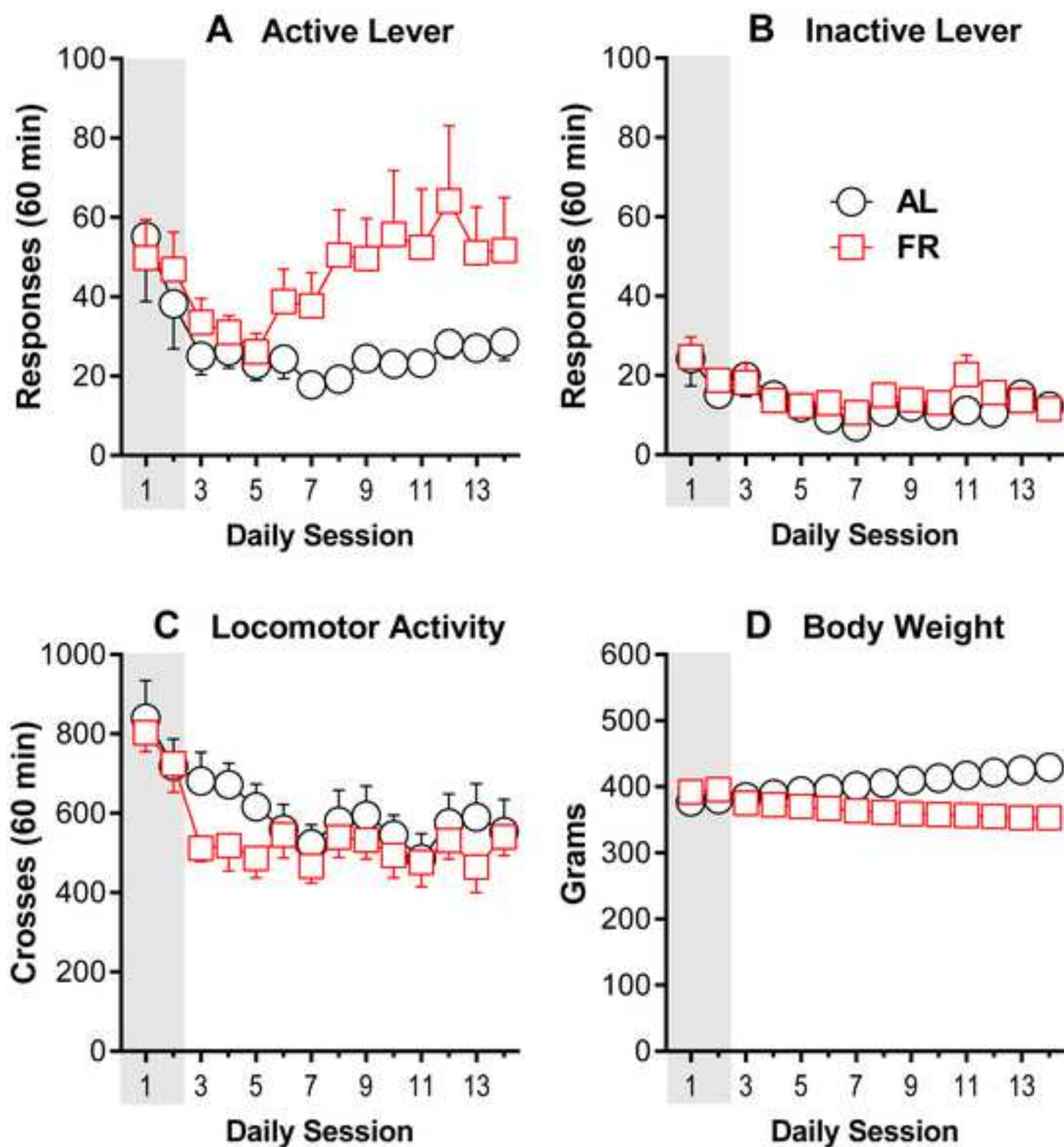
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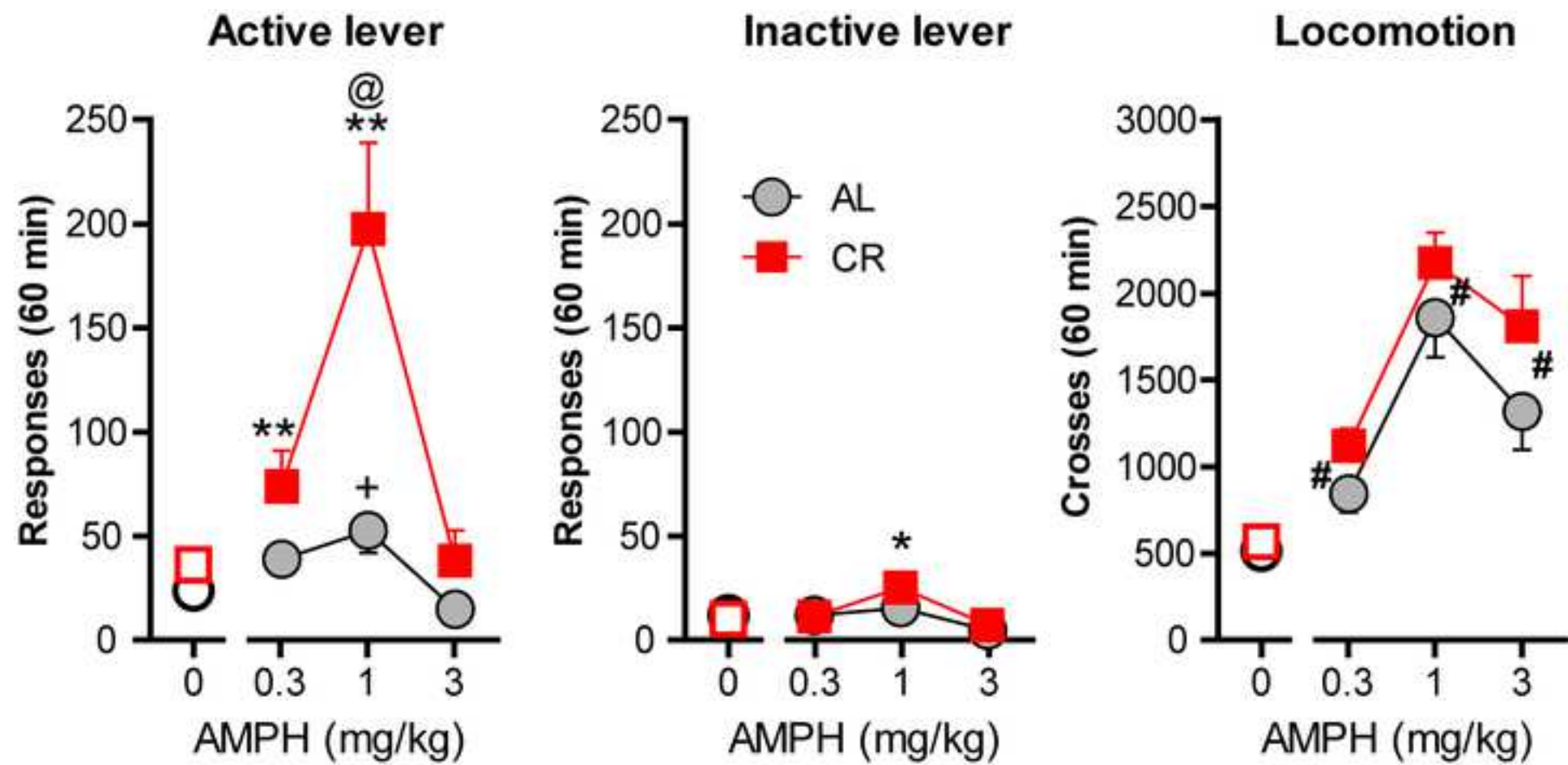
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Figure

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Name of Material/ Equipment	Company	Catalog Number	Comments/Description
			Operant conditioning
			chambers with levers and
Deluxe behavioral test package	Med Associates	MED-008-D1	stimulus lights
chamber modification for photocells	Med Associates	Env-008-PH	component of activity detection system
I/R cotroller	Med Associates	ENV-253	component of activity detection system
Infrared source and detector pair	Med Associates	ENV-253SD	component of activity detection system
Computer	Any PC computer		to control operant chamber hardware and recoi

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