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Journal

Psychopharmacology, 233(10)

Authors

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Publication Date

2016-05-01

DOI

10.1007/s00213-016-4222-0

Peer reviewed



Published in final edited form as:

Psychopharmacology (Berl). 2016 May ; 233(10): 1911–1919. doi:10.1007/s00213-016-4222-0.

A role for the endocannabinoid 2-arachidonoyl-*sn*-glycerol for social and high-fat food reward in male mice

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Abstract

Rationale—The endocannabinoid system is an important modulator of brain reward signaling. Investigations have focused on cannabinoid (CB₁) receptors, because dissection of specific contributions of individual endocannabinoids has been limited by the available toolset. While we recently described an important role for the endocannabinoid anandamide in the regulation of social reward, it remains to be determined whether the other major endocannabinoid, 2-arachidonoyl-*sn*-glycerol (2-AG), serves a similar or different function.

Objectives—To study the role of 2-AG in natural reward, we used a transgenic mouse model (MGL-Tg mice) in which forebrain 2-AG levels are selectively reduced. We complemented behavioral analysis with measurements of brain 2-AG levels.

Methods—We tested male MGL-Tg mice in conditioned place preference tasks (CPP) for high-fat food, social contact and cocaine. We measured 2-AG content in brain regions of interest by liquid chromatography/mass spectrometry.

Results—Male MGL-Tg mice are impaired in developing CPP for high-fat food and social interaction, but do develop CPP for cocaine. Furthermore, compared to isolated mice, levels of 2-AG in socially stimulated wild-type mice are higher in the nucleus accumbens and ventral hippocampus (183% and 140% of controls, respectively), but unchanged in the medial prefrontal cortex.

Conclusions—The results suggest that reducing 2-AG-mediated endocannabinoid signaling impairs social and high-fat food reward in male mice, and that social stimulation mobilizes 2-AG in key brain regions implicated in the control of motivated behavior. The time course of this

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Conflict of interest statement

The authors declare no conflict of interest.

response differentiates 2-AG from anandamide, whose role in mediating social reward was previously documented.

Keywords

monoacylglycerol lipase; social reward; high-fat reward

Introduction

The use of marijuana is reinforced through activation of the mesolimbic reward circuit (Gardner, 2005). In a related but distinct modulatory process, the neurotransmitter system mediating the effects of marijuana in the brain – the endocannabinoid system – also facilitates the reward of other stimuli, such as food or drugs of abuse (Solinas *et al.*, 2008; Maldonado *et al.*, 2006). The endocannabinoid system has three main components: (i) two lipid-derived local messengers – 2-arachidonoyl-*sn*-glycerol (2-AG) and anandamide (AEA), (ii) enzymes and transporters that are responsible for their formation and elimination, and (iii) receptors that are activated by endocannabinoids and regulate neuronal activity (Piomelli, 2014; Mechoulam and Parker, 2013).

Genetic and pharmacological studies have unveiled key roles of the CB₁ cannabinoid receptor in the modulation of reward-based behaviors. Less is known about the functions served by individual endocannabinoid messengers. In particular, an emerging question is whether endocannabinoids might also regulate the reward of social interactions. We recently demonstrated that anandamide regulates social reward via cooperative signaling with oxytocin, a neuropeptide that is crucial for social bonding (Wei *et al.*, 2015). The role of 2-AG remains unknown, however.

One way to assess the specific contribution of individual endocannabinoids is to manipulate the enzymes responsible for their formation and deactivation. For example, pharmacological inhibition or genetic deletion of the enzyme that hydrolyzes anandamide, fatty acid amide hydrolase (FAAH), markedly increases anandamide activity at CB₁ receptors (Cravatt *et al.*, 2001, Kathuria *et al.*, 2003). Analogous strategies exist for 2-AG. Indeed, MGL^{-/-} mice, in which the 2-AG-hydrolyzing enzyme monoacylglycerol lipase (MGL) is deleted, and thus 2-AG levels are elevated, as well as DGL- α ^{-/-} mice, in which the 2-AG-synthesizing enzyme diacylglycerol lipase (DGL- α) is deleted, and thus 2-AG levels are very low (Gao *et al.*, 2010; Chanda *et al.*, 2010). However, the effects of these radical modifications are often difficult to interpret because of the emergence of profound compensatory changes in the brain, such as desensitization of CB₁ receptors and elevation in anandamide and arachidonic acid levels (Gao *et al.*, 2010; Chanda *et al.*, 2010; Schlosburg *et al.*, 2010).

We have recently generated a novel transgenic mouse model – MGL-Tg mice – which selectively overexpress MGL in forebrain neurons under the control of the CaMKII promoter (Jung *et al.*, 2012). These mutant mice display a forebrain-selective accrual in MGL hydrolyzing activity and a 50–75% decrement in 2-AG content. This reduction in 2-AG is not accompanied by overt changes in levels of other endocannabinoid-related lipids (anandamide, arachidonic acid), cannabinoid receptors, or other endocannabinoid-related proteins (Jung *et al.*, 2012).

To investigate the role of 2-AG in reward-related behaviors, we tested male MGL-Tg mice in conditioned place preference (CPP) paradigms for high-fat food, social, or cocaine stimuli. Based on a rich theoretical framework, CPP assesses the rewarding value of test stimuli by pairing them with neutral environments (Bardo et al., 2000). Because less is known about endocannabinoid signaling and social behavior, we also investigated the effects of social interaction on 2-AG signaling in reward-related regions of the brain. We hypothesized that MGL-Tg mice might be deficient in reward signaling and that rewarding social stimuli drive 2-AG signaling in normal mice.

Materials and Methods

Animals

Male mice (6–10 weeks) were used. They were weaned at postnatal day 21 and group-reared. All testing was conducted during the light cycle (on at 0630 h and off at 1830 h). All procedures met the National Institute of Health guidelines for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee at University of California, Irvine.

Socially conditioned place preference (sCPP)—Procedures were previously described (Wei *et al.*, 2015). Briefly, mice were placed in a two-chambered acrylic box (27.3 × 27.3 cm). A 30-min pre-conditioning session was used to establish baseline neutral preference to two types of autoclaved, novel bedding (Alpha Dry, PharmaServ, Framingham, MA; and Kaytee Soft Granules, Petco, Irvine, CA). These differed in texture and shade (white vs. dark brown). Individual mice with strong baseline preference for either type of bedding were excluded – typically, those that spent more than 1.5× time on one bedding over the other. The next day, animals were randomly assigned to a social cage with cage-mates to be conditioned to one type of novel bedding for 24 h (in an unbiased, counterbalanced manner), then moved to an isolated cage with the other type of bedding for 24 h. On the next day, animals were tested alone for 30 min in the two-chambered box to determine post-conditioning preference for either type of bedding. Bedding volumes were 300 mL in each side of the two-chambered box and 550 mL in the home-cage. Familiar animals from the same cage were tested concurrently in four adjacent, opaque CPP boxes. Between trials, boxes were thoroughly cleaned with SCOE 10X odor eliminator (BioFOG, Alpharetta, GA). Scoring was automated using a validated image analysis script in ImageJ (Wei *et al.*, 2015).

Cocaine and high-fat diet CPP—Procedures were previously described (Wei *et al.*, 2015). Briefly, these paradigms were similar to social CPP, including unbiased and counterbalanced design, cleaning and habituation, exclusion criteria, and scoring, except for the following main differences, which followed reported methods (Bardo *et al.*, 2000; Perello *et al.*, 2010). Mice were conditioned and tested in a two-chambered acrylic box (31.5 × 15 cm). Pre- and post-conditioning tests allowed free access to both chambers and each had durations of 15 min (cocaine) and 20 min (high-fat). For conditioning, animals underwent 30-min sessions alternating each day between saline/cocaine (8 sessions total, 4 each) or standard chow pellet/high-fat pellet (12 sessions total, 6 each). The two chambers offered conditioning environments that differed in floor texture and wall pattern – sparse

metal bars on the floor and solid black walls vs. dense-wire-mesh floors and striped walls. Sparse metal bars allowed for paw access to the smooth acrylic floor, whereas dense-wire mesh did not. For high-fat diet CPP, animals were given one pellet of standard chow and an isocaloric amount of high-fat food (Research Diets, Inc., New Brunswick, NJ). As high-fat pellets have a different color and consistency, they were also given to home cages the day before pre-conditioning to prevent neophobia.

High-fat food intake

Intake of high-fat pellets (Research Diets, Inc., New Brunswick, NJ) was recorded in free-feeding mice using an automated monitoring system (Scipro, New York, NY, USA), as described previously (Gaetani *et al.*, 2003). Food intake was measured for two days, and the average of intake was normalized to the body weight at the start of feeding.

Social interaction test

The test was conducted according to established methods (File and Seth, 2003). To mimic the conditions of the social CPP task, mice were first isolated for 30 min and tested in dim-light conditions (5 lux). Pairs of mice were tested in an open field arena (50 × 50 × 40 cm) for 5 min. Scoring for social interaction time included behaviors such as sniffing, following, grooming, mounting and crawling over or under. Passive interaction, in which mice were in close proximity but without these interactions, was not included in the scoring.

Three-chambered social approach task

The procedure was previously described (Wei *et al.*, 2015), which was based on an established protocol (McFarlane *et al.*, 2008; Silverman *et al.*, 2012). Briefly, test mice were first habituated to an empty three-chambered acrylic box (40.6 × 21.6 cm), including to the center chamber for 10 min, and then to all chambers for 10 additional min. Mice were then tested for 10 min. Subjects were offered a choice between a novel object and a novel mouse in opposing side-chambers. The novel object was an empty inverted pencil cup and the novel social stimulus mouse was a sex, age and weight-matched 129/SvImJ mouse. These mice were used because they are relatively inert. They were trained to prevent erratic or aggressive behaviors, such as biting the cup. Weighted cups were placed on top of the pencil cups to prevent climbing. Low lighting (5 lux) was used. The apparatus was thoroughly cleaned with SCOE 10X odor eliminator (BioFOG) between trials to preclude olfactory confounders. Object/mouse side placement was counterbalanced between trials. Chamber time scoring was automated using image analysis. Sniffing time was scored by trained assistants who were unaware of treatment conditions. Outliers in inactivity or side preference were excluded.

Brain micropunches—The procedure was previously described (Wei *et al.*, 2015). Briefly, whole brains were collected and flash-frozen in isopentane at –50 to –60 °C. Frozen brains were transferred to –20°C in a cryostat and kept for 1 h to attain local temperature. The brain was then cut to the desired coronal depth and micropunches from bilateral regions of interest were collected using a 1×1.5-mm puncher (Kopf Instruments, Tujunga, CA). The micropunches weighed approximately 1.75 mg. A reference micropunch was taken to

normalize each punch to the brain's weight. Bilateral punches were combined for lipid analyses.

Lipid analyses

Procedures were previously described (Astarita and Piomelli, 2009; Wei *et al.*, 2015). Briefly, tissue samples were homogenized in methanol containing internal standards for H²-anandamide (H²-AEA), H²-oleoylethanolamide (H²-OEA) and ²H₈-2-arachidonoyl-*sn*-glycerol (²H₈-2-AG) (Cayman Chemicals, Ann Arbor, MI). Lipids were separated by a modified Folch-Pi method using chloroform/methanol/water (2:1:1) and open-bed silica column chromatography. For LC/MS analyses, we used an 1100 liquid chromatography system coupled to a 1946D-mass spectrometer detector equipped with an electrospray ionization (ESI) interface (Agilent Technologies, Palo Alto, CA). The column was a ZORBAX Eclipse XDB-C18 (2.1×100 mm, 1.8 μm, Agilent Technologies). We used a gradient elution method as follows: solvent A consisted of water with 0.1% formic acid, and Solvent B consisted of acetonitrile with 0.1% formic acid. The separation method used a flow rate of 0.3 mL/min. The gradient was 65% B for 15 min, then increased to 100% B in 1 min and kept at 100% B for 14 min. The column temperature was 15°C. Under these conditions, Na⁺ adducts of anandamide/H²-anandamide had retention times (Rt) of 6.9/6.8 min and *m/z* of 348/352, OEA/H²-OEA had Rt 12.7/12.6 min and *m/z* 326/330, and 2-AG/²H₈-2-AG had Rt 12.4/12.0 min and *m/z* 401/409. An isotope-dilution method was used for quantification.

Statistical analyses—Results are expressed as means ± SEM. Significance was determined by two-tailed Student's *t*-test, One-way or Two-way analysis of variance (ANOVA) with Tukey's post-hoc test. Differences were considered significant if *P*<0.05. Analyses were conducted using GraphPad Prism (GraphPad Software).

Results

MGL-Tg mice do not acquire CPP for high-fat food or social interaction

MGL-Tg mice eat less chow (either standard or high-fat) than do their wild-type littermates (Jung *et al.*, 2012). The food intake phenotype, however, does not dissociate the effects of 2-AG signaling on metabolic and reinforcement processes. Furthermore, altered feeding can be interpreted as either decreased or increased reward to the food stimulus. To isolate the effects of reduced 2-AG signaling on reward, we tested MGL-Tg mice and their wild-type (WT) littermates (C57Bl6J) in a CPP task for high-fat food. In a standard CPP box, mice were conditioned for 30-min sessions to either standard chow or isocaloric high-fat food for 6 sessions each, alternating over 12 days total (Perello *et al.*, 2010). In WT mice, we found that this conditioning protocol was sufficient to elicit a preference for the high-fat-paired chamber during post-conditioning testing. Animals spent 137 seconds more in the high-fat chamber compared to the standard-chow chamber (Fig. 1a-d). In contrast, MGL-Tg mice did not develop a preference for either chamber (Fig. 1a-d). This result suggests that 2-AG signaling is involved in conditioned reward processes of high-fat food.

We then asked whether this role for 2-AG signaling could extend to the reward produced by social interaction (Panksepp *et al.*, 2007; Pearson *et al.*, 2012; Dölen *et al.*, 2013). We conditioned mice for 24 h with cage-mates in their home-cage to one type of bedding, then we conditioned them for 24 h isolated to another bedding (Dölen *et al.*, 2013). In the post-conditioning test in a standard CPP box, we found that this conditioning was sufficient to elicit a preference in WT mice for the social bedding (Fig. 1e-h). In contrast, MGL-Tg mice did not develop a preference for either bedding (Fig. 1e-h). Together with the high-fat-food CPP results, these results suggest that 2-AG signaling may underlie aspects of reward processes common to both natural stimuli.

MGL-Tg mice show decreased intake of high-fat food but normal social interactions

The lack of CPP can be attributed to impairments in the generation and processing of the reward, the consolidation of the memory for the reward, or a combination of these two processes. To evaluate whether high-fat food stimuli are generated and processed properly, we measured initial intake of high-fat pellets over 2 days. MGL-Tg mice show a 16% reduction in normalized intake compared to WT littermates over this period (Fig. 2a). The combined phenotype of MGL-Tg mice showing a lack of CPP and decreased intake suggests that 2-AG plays a role in the generation and processing of high-fat food reward. A strict interpretation of these results may be complicated, however, by the role of 2-AG in energy metabolism (Jung *et al.*, 2012).

For the same reason, we also examined the direct social activity and the social approach interest of MGL-Tg mice using the social interaction test (File and Seth, 2003) and the three-chambered social approach test (McFarlane *et al.*, 2008; Silverman *et al.*, 2012), respectively. These tests differ in two key ways: (i) the social interaction test evaluates interactions that are reciprocal and direct, whereas the social approach test measures approach activity to a stimulus mouse that is sequestered in an inverted wire cup; (ii) the social interaction test uses familiar cage-mates, whereas the social approach test uses a novel mouse as a stimulus. In the social interaction test, we observed that MGL-Tg mice trend toward less interaction time, but this result was not significant (Fig. 2b). In the social approach test, we found that both MGL-Tg and WT mice preferred the social chamber over the object chamber and sniffed the stimulus mouse more than the object (Fig. 2c,d). MGL-Tg mice were similar to WT mice in the amount of time spent in the social chamber and sniffing the stimulus mouse (Fig. 2c,d). These results suggest that MGL-Tg mice show similar levels of social interaction and social interest as WT mice, regardless of the familiarity or the reciprocity of the social stimulus. Therefore, in contrast to high-fat food, the impaired CPP of MGL-Tg mice to social interactions may represent a problem of consolidation rather than reward processing.

MGL-Tg mice acquire CPP to cocaine

It has been postulated that drugs of abuse ‘hijack’ natural reward circuits (Gardner, 2005; Fattore *et al.*, 2010). We therefore tested MGL-Tg mice in cocaine CPP to see if the drug might recruit 2-AG signaling. In a standard CPP box, we conditioned mice to either saline or cocaine (5 mg·kg⁻¹, intraperitoneal) in four 30-min sessions, alternating over a total of 8 days (Bardo *et al.*, 2000). Both WT and MGL-Tg mice developed a strong post-conditioning

preference for the cocaine chamber (Fig. 3a-d). This result suggests that the conditioned effects of cocaine do not depend on 2-AG signaling. Furthermore, it indicates that MGL-Tg mice are cognitively able to perform CPP in general.

Social stimulation increases 2-AG

Previous work has linked feeding status and social contact with brain endocannabinoid levels (Di Marzo *et al.*, 2001; Kirkham *et al.*, 2002; Wei *et al.*, 2015). In particular, we have recently shown that social contact increases anandamide mobilization in the NAc, and that this response plays an important role in social reward (Wei *et al.*, 2015). If 2-AG is also involved in social-reward processes, as our previous results suggest, social stimulation should increase 2-AG levels. We isolated male juvenile C57Bl6J mice for 24 h, and then returned half to their cage-mates while keeping the other half isolated for 6 h (Fig. 4a). We collected and snap-froze their brains, took micro-punches of tissue, and measured lipid content using liquid chromatography-mass spectrometry (LC/MS). We found that, compared to mice that remained isolated, socially stimulated mice showed an 83% increase in 2-AG levels in the nucleus accumbens (NAc) and a 40% increase in the ventral hippocampus (vHC), but did not show a change in 2-AG levels in the medial prefrontal cortex (mPFC) (Fig. 4b-d). This suggests that social interactions cause a mobilization of 2-AG signaling. Levels of the endocannabinoid anandamide, in contrast, were unchanged in the NAc after this 6-h social stimulation (Fig. 4e). It is important to note, however, that we previously observed a different pattern for a 3-h social stimulation, which elicited an increase in levels of anandamide but not 2-AG (Wei *et al.*, 2015).

It is possible that social contact elevates 2-AG levels due to an increase in general activity. Therefore, we compared 24-h-isolated animals to control animals that were similarly handled but remained social (Fig. 4f). In this comparison, isolation had no effect on levels of 2-AG in the NAc, but decreased 2-AG by 55% in the vHC and by 50% in the mPFC (Fig. 4g-i). This profile of 2-AG change was not the opposite of that elicited by social stimulation – that is, NAc 2-AG increased during stimulation but did not change during isolation, and mPFC did not change during stimulation but decreased during isolation. In contrast, the pattern of 2-AG levels was opposite in the vHC – 2-AG increased during stimulation and decreased during isolation. The magnitudes of these opposing effects in the vHC were relatively comparable (40% increase and 55% decrease). Isolation also did not alter levels of anandamide in the NAc (Fig. 4j).

We interpret these results to mean that social stimulation induces 2-AG signaling in the NAc that is distinct from what is active during baseline social activity, i.e. a ‘social 2-AG tone.’ Other contributions in the mPFC and vHC remain to be determined. Nevertheless, because the NAc is a key region for brain reward, the results suggest that 2-AG likely contributes to the reward of social interactions. These results support the previous finding that MGL-Tg are deficient in social CPP.

Discussion

It has been postulated that the signaling system underlying reward for social interactions overlaps with those involved in the control of other natural rewards (Panksepp *et al.*, 1997).

The available literature emphasizes the important role of the endocannabinoid system in motivated behaviors (Fattore *et al.*, 2010; Maldonado *et al.*, 2006; Solinas *et al.*, 2008; Parsons and Hurd, 2015), raising the theoretical possibility of an endocannabinoid substrate that is common to different natural rewards. Our study provides evidence in support of this possibility through complementary behavioral analysis of a unique model of selective 2-AG reduction with biochemical analysis of 2-AG mobilization in brain regions important for reward. Our main findings are that 2-AG reduction impairs CPP for social interactions, and that social stimulation increases 2-AG levels in the NAc. These results clearly identify 2-AG as a reward signal for social interaction. Additionally, we found that 2-AG reduction impairs CPP to high-fat food. Collectively, our results suggest that 2-AG is involved in regulating the incentive salience of two essential aspects of behavior, feeding and social contact.

A limited number of studies have addressed the involvement of endocannabinoid signaling in the control of social behavior. Genetic CB₁ deletion and administration of cannabinoid agonists alter social interactions. The effect of cannabinoid agonists can be bidirectional, depending on dosage and thus likely the competing circuits involved (Haller *et al.*, 2004; Trezza and Vanderschuren, 2008). Furthermore, increasing anandamide activity via genetic deletion or inhibition of its hydrolytic enzyme, FAAH, increases direct social interactions in mice and social play in rats (Cassano *et al.*, 2011; Trezza and Vanderschuren, 2008). Recently, we demonstrated that anandamide-mediated endocannabinoid signaling is important in the control of social reward (Wei *et al.*, 2015). Social contact mobilizes anandamide in an oxytocin receptor-dependent manner. Consistently, chemogenetic activation of oxytocin neurons in the hypothalamus increases anandamide mobilization in the NAc. Pharmacological and genetic enhancement of anandamide activity (i.e. with FAAH inhibitors or genetic FAAH deletion) increases social CPP and offsets the effects of oxytocin blockade. These results suggest that a cooperative oxytocin-driven anandamide signal regulates social reward (Wei *et al.*, 2015).

Thus, the distinct effects of cannabinoid agonists and FAAH inhibitors on social behavior present a complex picture with two knowledge gaps: (i) whether 2-AG-mediated activation of CB₁ receptors also participates in social behaviors; and (ii) if so, the type of interaction and associated neural circuits that are regulated. The present study addresses these gaps by (i) exploiting a new transgenic mouse to reduce endogenous 2-AG signaling without overt compensation, (ii) using CPP to specifically model social reward rather than interaction in general, and (iii) measuring socially mobilized 2-AG levels in brain areas that are part of the reward circuit.

At the same time, our study raises an important question for future studies. We found that the temporal profiles of socially stimulated 2-AG versus anandamide are distinct. In the NAc, a 3-h social stimulation increases the levels of anandamide, but not 2-AG (Wei *et al.*, 2015), whereas a 6-h stimulation elevates levels of 2-AG, but not anandamide (Fig. 4b,e). Distinct temporal profiles of endocannabinoid mobilization have been demonstrated in other situations – for example, in response to drugs of abuse (Solinas *et al.*, 2008) as well as in stress-induced analgesia mediated by the periaqueductal grey (Hohmann *et al.*, 2005) – and thus are likely to be functionally meaningful. One plausible explanation is that anandamide is involved in the initial saliency of a social encounter whereas 2-AG is involved in

consolidating information from prolonged social contact. This hypothesis is supported by two pieces of evidence. First, oxytocin is thought of as a saliency signal that is more proximal to the initial processing of social sensory information (Insel, 2010; Young and Barrett, 2015). Oxytocin tightly drives anandamide formation in the NAc, whereas 2-AG production is driven by prolonged social contact, but not oxytocin (Wei *et al.*, 2015; Fig. 4b,c). Secondly, anandamide is more specific to social reward whereas 2-AG is more generalizable to other rewards, as elevating anandamide increases CPP for social interactions but not for high-fat food or cocaine (Wei *et al.*, 2015), whereas reducing 2-AG activity reduces CPP to both social interactions and high-fat food (Fig. 1).

An extensive literature also supports an important role of CB₁ signaling in food reward. CB₁ receptor antagonism and genetic deletion suppress not only food intake, but also CPP and self-administration (Arnone *et al.*, 1997; Sanchis-Segura *et al.*, 2004; Solinas and Goldberg, 2005). In contrast, information is sparse regarding the individual endocannabinoid transmitters that might be involved. Published studies have found that systemic administration of exogenous anandamide increases food intake (Williams and Kirkham, 1999), and 2-AG administration into the NAc increases feeding (Kirkham *et al.*, 2002). Exogenous administration, however, does not simulate physiological conditions. The intake phenotype may also equivocate the metabolic and motivational aspects of endocannabinoid signaling. Our study addresses these concerns by specifically manipulating 2-AG signaling and using a model of CPP to represent the rewarding value of high-fat food.

A lack of CPP encompasses different impairments in aspects of reward signaling, such as the processing of initial sensory cues, integration and recruitment of limbic regions, and the consolidation of the memory for the reward. In order to specify the component in which 2-AG may play a larger role, we also measured the intake of high-fat food and the social interactions of MGL-Tg mice. We found that MGL-Tg mice show less intake of high-fat food but appear normal in direct, reciprocal social interactions as well as social approach. In light of the lack of CPP for both high-fat and social stimuli in MGL-Tg mice, these results may point to a dichotomous role for 2-AG – perhaps in the processing of high-fat reward versus the consolidation of social reward. These speculations are consistent with the aforementioned feeding literature and prolonged action of 2-AG, as compared to anandamide, after social stimulation. In line with this thinking, the phenotype of a lack of CPP across high-fat and social stimuli, but present CPP for cocaine, must be taken to represent a qualitative rather than quantitative difference. That is, reducing 2-AG impairs different processes for the reward signaling of these stimuli, rather than different amounts of the same process. Perhaps because cocaine bypasses 2-AG-regulated signaling and is more dopamine-dependent, CPP develops regardless of 2-AG signaling. Nevertheless, whether 2-AG influences dopamine in MGL-Tg mice and whether dopamine is the ultimate effector of all these rewards remains to be determined. Indeed, the magnitude of cocaine CPP as a function of dosage does not vary in a graded manner, but develops in an all-or-none fashion, starting at the minimum dose range that we used (5 mg·kg⁻¹, i.p.) (Brabant *et al.*, 2005). Additional investigation into these possibilities is needed.

While MGL-Tg mice represent a technological advance to address the aforementioned knowledge gaps, phenotypic results also come with potential weaknesses. First, forebrain 2-

AG reduction may alter general cognition so that mice are unable to perform the CPP task. However, our finding that MGL-Tg mice develop normal cocaine CPP argues against this possibility. Second, improper development or socialization may render MGL-Tg abnormal. But this is unlikely because MGL overexpression is controlled by the CAMKII promoter, which lacks developmental activity, and MGL-Tg mice do not show overt abnormalities in tests of general health (Jung *et al.*, 2012). Normal levels of social interaction and social approach also speak to proper socialization. It remains true that our results do not completely exclude these possibilities. More nuanced forms of cognition or social interaction may not be detected in our tests.

In conclusion, our study identifies a common endocannabinoid substrate – 2-AG – in the regulation of two natural rewards, feeding and social contact. This identification provides a basis to motivate further investigation of the circuitry and physiology regulated by 2-AG signaling in natural reward, the dichotomous roles of anandamide and 2-AG in different social contexts, as well as how such signaling may be exploited in addiction.

Acknowledgments

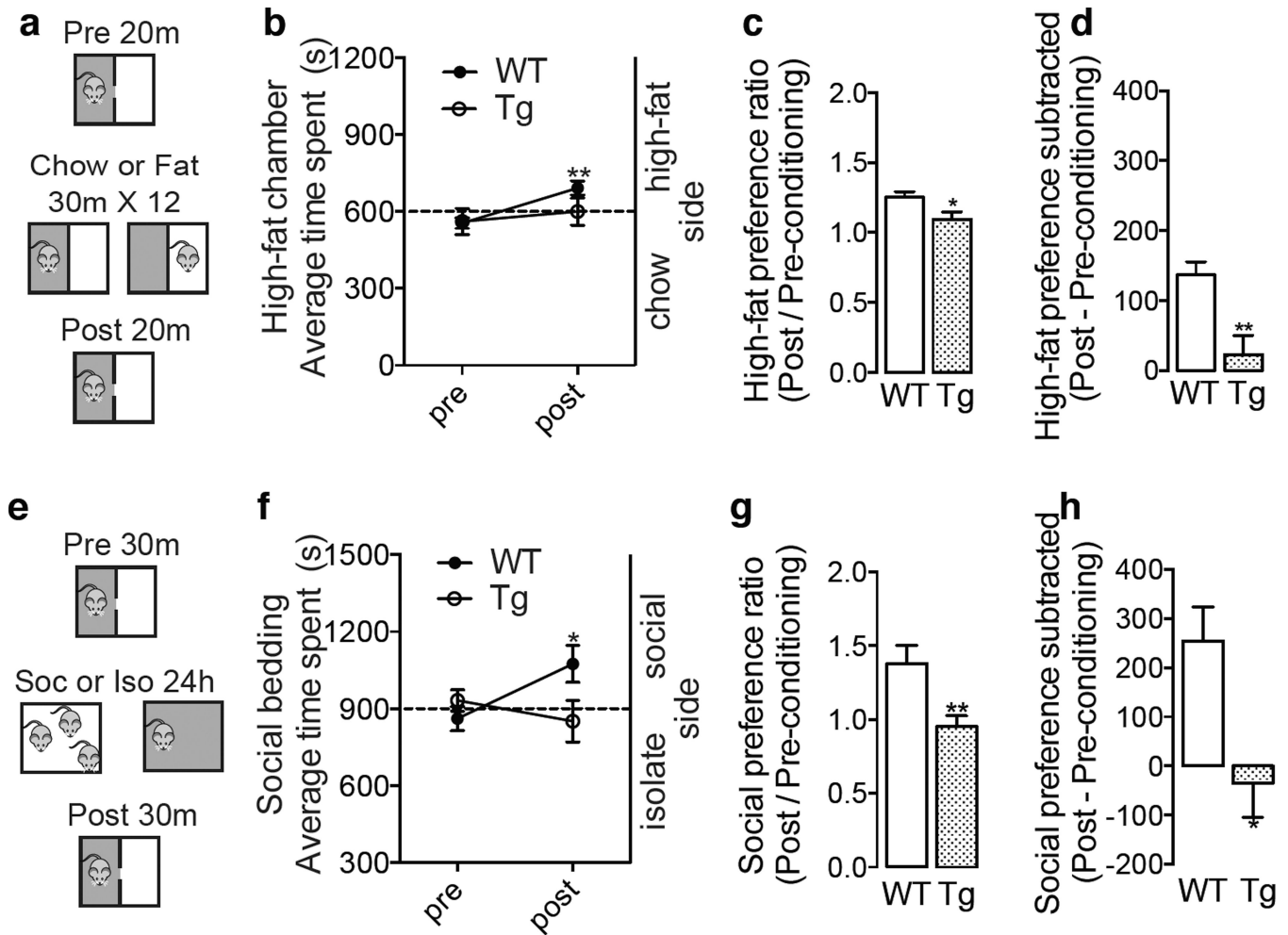
The authors thank Terri Wang for her generous gift. The authors thank the Autism Science Foundation, the UC Irvine Center for Autism Research and Translation, and the UC Irvine Medical Scientist Training Program.

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**Fig. 1.**

MGL-Tg mice do not acquire CPPs to high-fat food and social interaction. (a) Schematic of conditioned place preference (CPP) task for high-fat food. (b) Time spent in high-fat chamber. (c) High-fat preference as a ratio of time spent in high-fat chamber during post-conditioning trial to pre-conditioning trial. (d) High-fat preference as a difference in time spent in high-fat chamber between post-conditioning and pre-conditioning trials. (e) Schematic of CPP task for social interaction. (f) Time spent in social chamber. (g) Social preference as a ratio of time spent in social chamber during post-conditioning trial to pre-conditioning trials. (h) Social preference as a difference in time spent in social chamber between post-conditioning and pre-conditioning trials. Results are expressed as mean \pm SEM; n=10–12 per group. Students *t* test, comparing pre- to post-conditioning (b,f), or WT to Tg (c-d, g-h). **P* < 0.05, ***P* < 0.01.

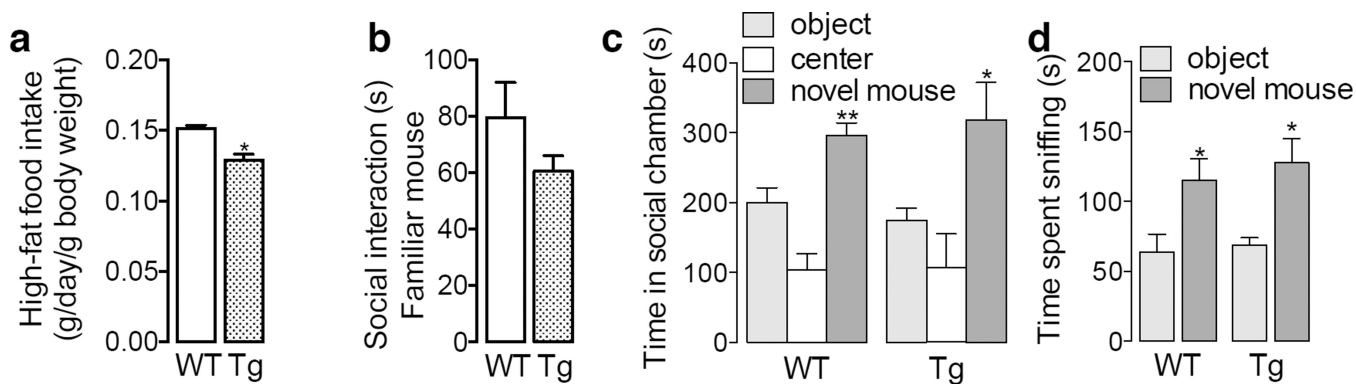


Fig. 2.

MGL-Tg mice show decreased high-fat intake but normal social interaction and social approach behavior. (a) High-fat food intake. The average of two days was normalized to the starting weight. (b) Social interaction time. Time spent in chambers of a three-chambered apparatus, in which a novel stimulus mouse is contained in a pencil cup (object) in one side chamber and the opposite side chamber contains only the same empty object. (c) Time spent sniffing the novel object or novel mouse. Results are expressed as mean \pm SEM; $n=4-5$ per group (a,b), $n=8$ per group (c,d). Students *t* test, comparing WT to Tg (a,b), or object to mouse time (c,d). * $P < 0.05$, ** $P < 0.01$.

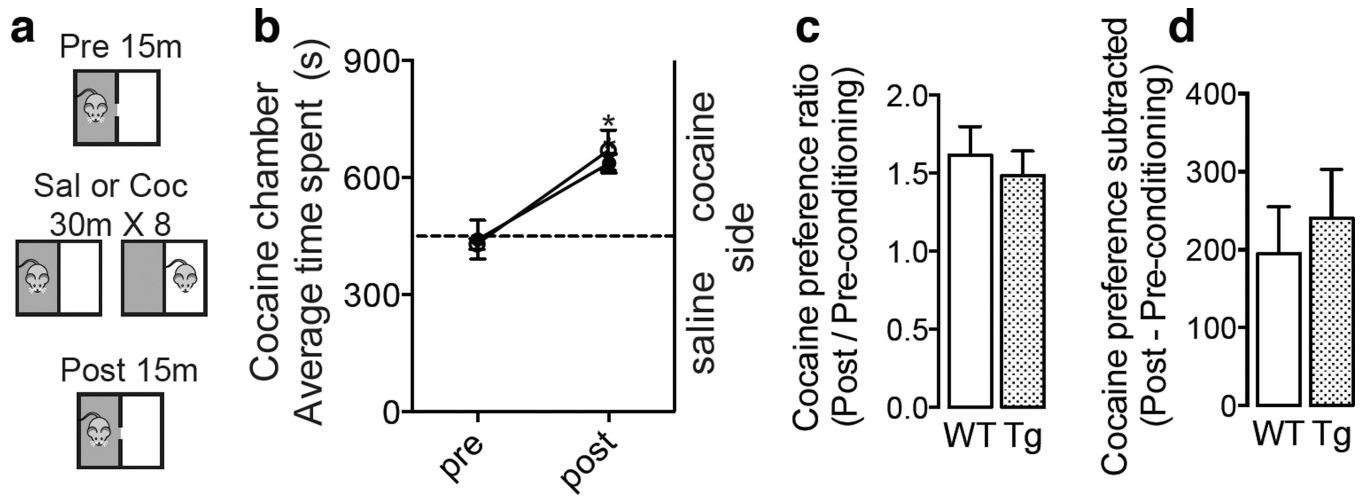


Fig. 3. MGL-Tg mice acquire CPP to cocaine. (a) Schematic of conditioned place preference (CPP) task for cocaine ($5 \text{ mg}\cdot\text{kg}^{-1}$, intraperitoneal). (b) Time spent in cocaine chamber. (c) Cocaine preference as a ratio of time spent in cocaine chamber during post-conditioning trial to pre-conditioning trial. (d) Cocaine preference as a difference in time spent in cocaine chamber between post-conditioning and pre-conditioning trials. Results are expressed as mean \pm SEM; $n=10$ per group. Students t test, comparing pre- to post-conditioning (b), or WT to Tg (c,d). * $P < 0.05$.

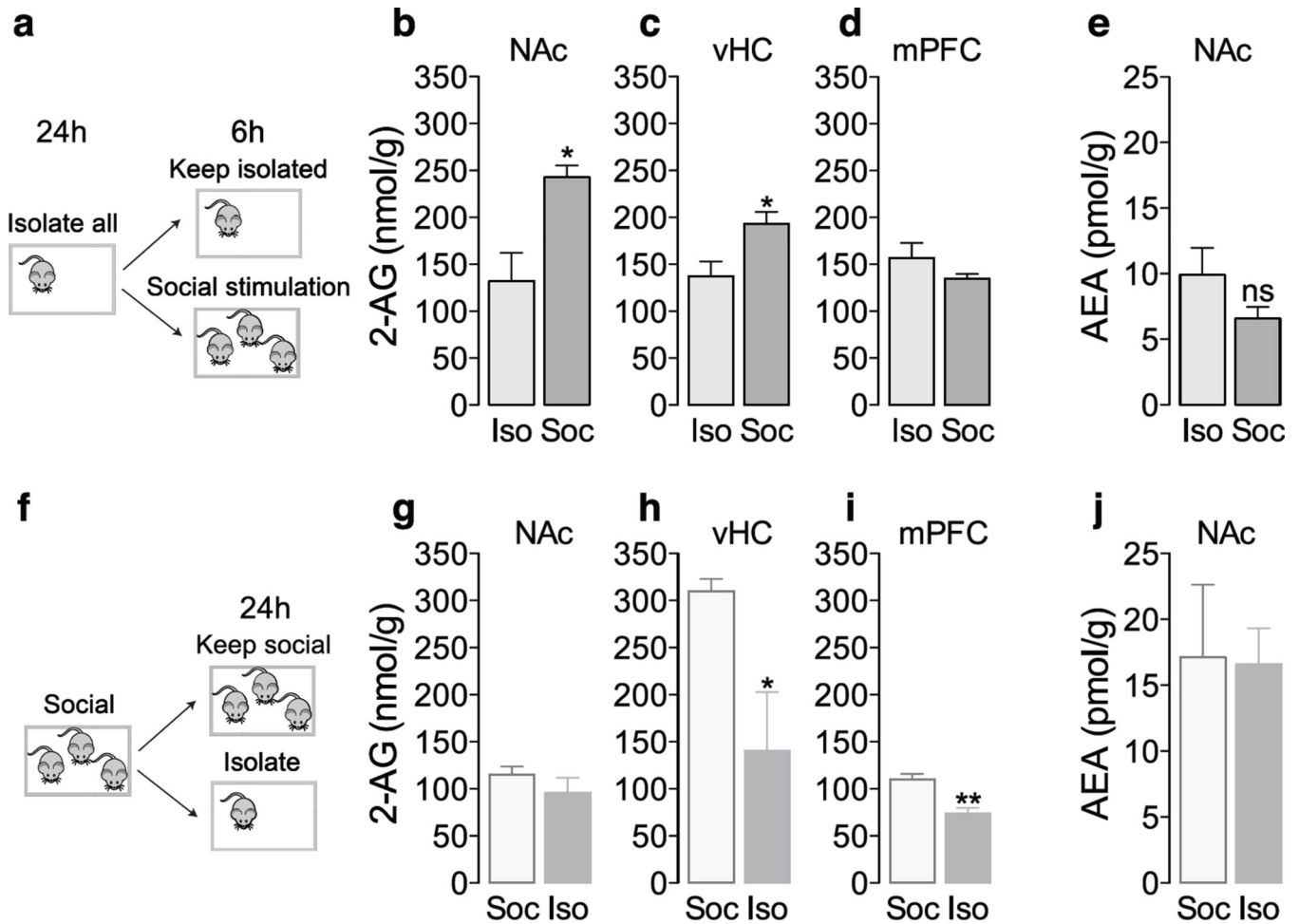


Fig. 4. Social stimulation increases 2-AG in C57B16J mice. (a) Schematic of social stimulation. (b-d) 2-AG levels in the nucleus accumbens (NAc), ventral-to-mid hippocampus (vHC), and medial prefrontal cortex (mPFC). (e) Levels of anandamide (AEA) in the NAc. (f) Schematic of isolation. (g-i) 2-AG levels in the NAc, vHC, mPFC. (j) Levels of anandamide (AEA) in the NAc. Results are expressed as mean \pm SEM; n=4-6 per group. Students *t* test. * $P < 0.05$, ** $P < 0.01$. ns = not significant.