Received: 25 January 2024 | Revised: 22 May 2024 | Accepted: 27 May 2024

DOI: 10.1111/adb.13420

ORIGINAL ARTICLE

Addiction Biology SSA WILEY

# Social setting interacts with hyper dopamine to boost the stimulant effect of ethanol

Dilean J. Murillo Gonzalez[1,2] | Bryan A. Hernandez Granados[1,3] | Paul Rafael Sabandal[1] | Kyung-An Han[1]

[1]Department of Biological Sciences, The University of Texas at El Paso, El Paso, TX, USA

[2]Department of Neuroscience, Baylor College of Medicine, Houston, TX, USA

[3]Department of Biochemistry, Vanderbilt University, Nashville, TN, USA

**Correspondence**
Kyung-An Han and Paul Rafael Sabandal, Department of Biological Sciences, The University of Texas at El Paso, El Paso, TX, 79968, USA.
Email: khan@utep.edu and pbsabandal@utep.edu

**Funding information**
Orville Edward Egbert, M.D. Endowment fund; NIH NIGMS 1R16GM145548; NIH NIMHD 3U54MD007592-29S5

## Abstract

Alcohol consumption occurring in a social or solitary setting often yields different behavioural responses in human subjects. For example, social drinking is associated with positive effects while solitary drinking is linked to negative effects. However, the neurobiological mechanism by which the social environment during alcohol intake impacts on behavioural responses remains poorly understood. We investigated whether distinct social environments affect behavioural responses to ethanol and the role of the dopamine system in this phenomenon in the fruit fly *Drosophila melanogaster*. The wild-type *Canton-S* (*CS*) flies showed higher locomotor response when exposed to ethanol in a group setting than a solitary setting, and there was no difference in females and males. Dopamine signalling is crucial for the locomotor stimulating effect of ethanol. When subjected to ethanol exposure alone, the dopamine transport mutant flies *fumin* (*fmn*) with hyper dopamine displayed the locomotor response similar to *CS*. When subjected to ethanol in a group setting, however, the *fmn*'s response to the locomotor stimulating effect was substantially augmented compared with *CS*, indicating synergistic interaction of dopamine signalling and social setting. To identify the dopamine signalling pathway important for the social effect, we examined the flies defective in individual dopamine receptors and found that the D1 receptor dDA1/Dop1R1 is the major receptor mediating the social effect. Taken together, this study underscores the influence of social context on the neural and behavioural responses to ethanol.

**KEYWORDS**
D1 receptor, dopamine, dopamine transporter, *Drosophila*, ethanol, hyperactivity, social context, social environment, social setting, stimulant effect

## 1 | INTRODUCTION

Alcohol is a commonly abused drug worldwide. Its consumption is influenced by many factors including genetics, stress, and social context.[1,2] For example, social drinking is associated with positive

Dilean J. Murillo Gonzalez and Bryan A. Hernandez Granados contributed equally to this work.

subjective effects such as euphoria, positive mood, elation, and friendliness,[3] whereas solitary drinking is related to depressive symptoms and suicidal ideation.[4] Both solitary and social drinking are associated with negative alcohol-related outcomes that are related to AUD likely via different mechanisms.[5,6] In rodent models, ethanol consumption alters social activity in a dose- and age-dependent manner[7,8] while social housing increases ethanol consumption and self-administration in rats, mice, and voles.[9–11] The mechanism by which social environment at the time of alcohol intake affects behavioural responses, however, remains highly understudied.

The fruit fly *Drosophila melanogaster* is a useful model for alcohol research.[12] In its natural environment, *Drosophila* prefers to lay eggs in fermented fruits containing ethanol.[13] At low ethanol doses, flies display hyperactivity, and at high doses, they show hypoactivity followed by sedation[14] similar to humans and rodents. As in rodents, the locomotor-stimulating effect of ethanol has been used to study euphoria.[14,15] Also as in humans and rodents, ethanol consumption alters social behaviours in flies in that ethanol intake increases sexual arousal, disinhibits inter-male courtship, and decreases sexual performance.[16] Prior social experiences also alter ethanol preference and responses. Unsuccessful mating augments ethanol intake in male flies[17] while social isolation during rearing decreases ethanol sensitivity to the sedative effect of ethanol.[18] There is no information, however, whether social environment at the time of ethanol intake affects the locomotor activating effect of ethanol.

Ethanol consumption influences the dopamine system. In humans and rodents, alcohol drinking increases dopamine release in the nucleus accumbens.[19,20] An increase in extracellular dopamine causes euphoric responses and is a key step for addiction.[21] Likewise, the ethanol-induced increase in dopamine signalling contributes to the development of AUD.[21–23] In flies, the dopamine system is important for ethanol effects as well.[24,25] The blockade of dopamine neurotransmission or D1 receptor mutation decreases ethanol-induced hyperactivity[25] while dopamine and ecdysone receptor (DopEcR) mutation dampens behavioural sensitization.[26] In this study, we investigated whether social context impacts the locomotor-stimulating effect of ethanol and whether dopamine influences this phenomenon in *Drosophila*.

## 2 | METHODS

### 2.1 | Fly strains and culture

The wild-type strain used in the study is *Canton-S* (*CS*). The dopamine transporter (DAT) mutant *fumin* (*fmn*) in which the *roo* transposon insertion in the sixth intron of the DAT gene causes premature termination[27] was obtained from Dr. Jackson (Tufts University, Boston, MA) and placed in the *CS* background. The dopamine receptor mutants used in this study were *dumb*$^{1}$ (*ln*$^{3RL}$*234*), *dumb*$^{2}$ (*f02676*) and *dumb*$^{4}$ (*MI04437*) defective in dDA1/Dop1R1, *d2r* (*f06521*) defective in dD2R/Dop2R,[26,28,29] *damb* defective in DAMB/Dop1R2,[30,31] and *der* defective in DopEcR (*c02142*).[26] These stocks were obtained from the Exelixis Collection (Harvard Medical School, Boston, MA, USA) and placed in the *CS* background. All flies used in this study have the wild-type *white* allele (*w*+).

Flies were raised on a standard sucrose/yeast agar medium (25°C) with 50–60% relative humidity under the 12-h light/12-h dark cycle. Flies were collected within 24 h of eclosion, housed in mixed-sex groups for 2 days in food bottles, anaesthetised using carbon dioxide, separated into either a group of 13–15 males or a group of 13–15 females in food vials, and then placed in a temperature- (25°C) and humidity-controlled (50–60%) incubator for 24–48 h before ethanol exposure.

### 2.2 | Ethanol exposure and behaviour measurement

A single fly or a group of flies (13-15) was placed in a plexiglass chamber (60 mm L × 60 mm W × 15 mm H) and acclimated to the chamber for 10 min and then to water vapour for 10 min followed by ethanol vapour exposure for 10 min as previously described.[24] The chamber was connected to the tubing delivering either humidified air for acclimation or humidified air combined with absolute ethanol for 40, 50, or 60% ethanol vapour, all at the 2.5 L/min flow rate. Basal and ethanol-induced behavioural responses were analysed using the Viewer3 software (BioObserve, Germany) that tracks and measures the walking speeds of individual flies in mm/sec. The walking speed was measured every second, and the highest average speed during the 30-s period after the startle response noted as a top speed was used for comparison of locomotor responses. A group of 13–15 flies represents $n = 1$ for the group-exposed condition, while one fly represent $n = 1$ for the singly exposed condition. Male and female data were analysed and presented separately.

### 2.3 | Data analysis

All statistical analyses were performed using Minitab 21 (Minitab, State College, PA, USA). All data are reported as mean ± standard error of the mean (SEM). Normality was determined by the Anderson Darling goodness-of-fit test. Normally distributed datasets were analysed by either two-tailed Student's *t* test or analysis of variance (ANOVA) *with* post hoc Tukey or Student's *t* test. Non-normally distributed datasets were analysed by Kruskal–Wallis and Mann–Whitney tests. A power analysis indicated that $n = 6$ was the required sample size to achieve 80% power for detecting the effect size of 0.5 at a significance level of $\alpha = 0.05$.

## 3 | RESULTS

### 3.1 | Social context affects ethanol-induced hyperactivity

To investigate how social settings at the time of ethanol intake affect behavioural responses, we exposed wild-type *CS* flies alone or in a group to ethanol. Consistent with previous reports,[25] introduction of the ethanol vapour induced startle response, which was followed by hyperlocomotor activity and then hypoactivity (Figure 1A,B). This

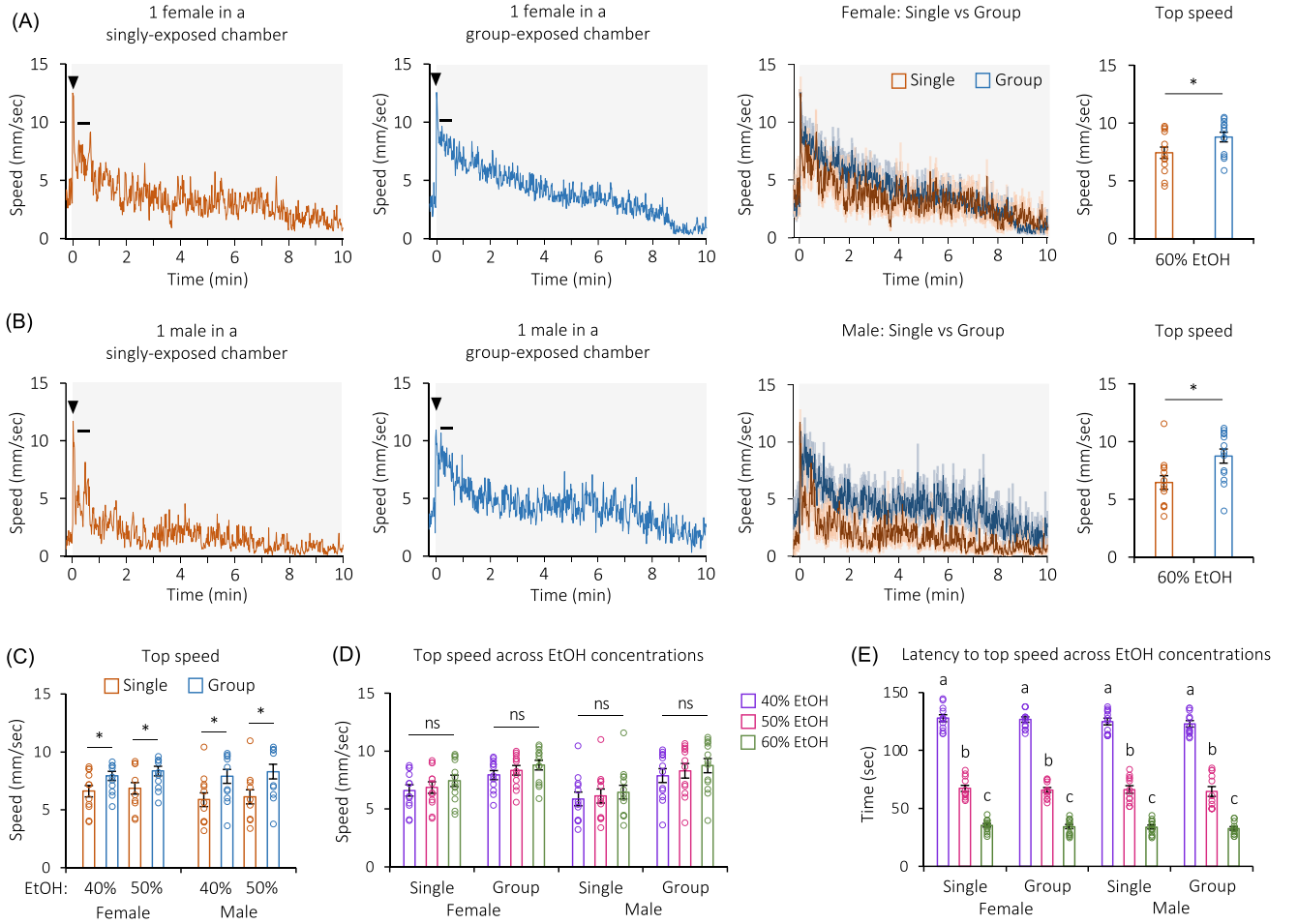


**FIGURE 1** Social context affects ethanol-induced hyperactivity. The wild-type *CS* flies were exposed to ethanol in a single (orange) or group setting (blue). Shown from left to right are a single fly's walking speed trace in a singly exposed chamber, a single fly trace in a group-exposed chamber, the average of multiple chambers per social setting with SEM in shaded colour, and top speeds (rightmost panel) of females (A) and males (B) exposed to 60% ethanol. Ethanol exposure started at 0 min. The walking speed traces include startle response when ethanol was first introduced in the chamber (black arrowhead), locomotor response to ethanol (0–10 min shaded in light grey), and the top speed (a 30-sec interval showing the maximal activity during ethanol exposure is denoted by a black line). In both sexes, group-exposed flies displayed higher locomotor responses to ethanol compared with singly exposed flies (*$p < 0.05$; $n = 13$). (C) the top speeds of female and male *CS* to either 40% or 50% ethanol in different social settings were compared. In both 40% and 50% ethanol concentrations, the group-exposed *CS* exhibited greater top speeds compared with singly exposed flies (*$p < 0.05$; $n = 12$). (D) the *CS* top speeds during exposure to various ethanol concentrations. The *CS* top speeds were not different across different ethanol concentrations (ns, not significant, $p > 0.05$; $n = 12–13$). (E) the *CS*'s latencies to the ethanol-induced top speeds. The latencies to the ethanol-induced top speeds showed a dose-dependent relationship, where higher ethanol doses led to shorter latencies to top speeds (different letters denote significant differences; $p < 0.05$; $n = 12–13$)

pattern of the ethanol-induced locomotor response was similar in both social settings and sexes. The magnitude of hyperlocomotor activity, however, was significantly different between single and group settings. To quantitatively compare the difference, we measured the average speed of the flies in 30-s duration and compared the highest average ("top speed") per setting (Figure 1A,B right panels, C,D). The group-exposed *CS* flies displayed significantly higher top speeds compared with singly exposed *CS* with all ethanol concentrations under test in both sexes (Figure 1A–C). We did not observe significant differences between sexes in either singly or group-exposed condition (two-way ANOVA: social setting, $F_{1,51} = 11.31$, $p = 0.002$; sex effect, $F_{1,51} = 0.89$, $p = 0.35$; social setting × sex interaction, $F_{1,51} = 0.76$, $p = 0.388$). Notably, the top speeds of three ethanol concentrations were comparable (Figure 1D; ANOVA; ns, $p > 0.05$, $n = 12–13$); however, their latencies were significantly different in that higher ethanol concentration caused faster onset of the top speed (Figure 1E; ANOVA; $p < 0.05$, $n = 12–13$). It is worth noting that the total activity under the influence of ethanol was significantly lower in the singly exposed males compared with other groups ($p < 0.0001$). In the absence of ethanol, the baseline activity of *CS* in all social settings and sexes was comparable (two-way ANOVA: social setting, $F_{1,51} = 0.06$, $p = 0.812$; sex effect, $F_{1,51} = 0.01$, $p = 0.905$; social setting × sex interaction, $F_{1,51} = 0.14$, $p = 0.709$). These results indicate that the flies exposed to ethanol in the presence of other flies display the augmented hyperlocomotor response and there is no difference between males and females in this phenomenon.

### 3.2 | Concurrent social enrichment and increased dopamine signalling amplify ethanol-induced hyperactivity

To explore whether dopamine plays a role in the social context-sensitive ethanol response, we tested the *fmn* mutant lacking DAT thus having hyper dopamine signalling.[32] Similar to *CS*, the group-exposed *fmn* showed the enhanced hyperlocomotor response compared with singly exposed *fmn* with all three ethanol concentrations and in both sexes (Figure 2A–C), in which there was no sex difference in either singly or group-exposed condition (two-way ANOVA: social setting, $F_{1,38} = 178.04$, $p < 0.0001$; sex effect, $F_{1,38} = 1.38$, $p = 0.248$; social setting × sex interaction, $F_{1,38} = 0.11$, $p = 0.747$). The *fmn*'s top speeds across different ethanol concentrations were also comparable (Figure 2D; ANOVA; ns, $p > 0.05$, $n = 9–10$), but their latencies were significantly different, where the faster top speed onsets correlated with higher ethanol concentrations (Figure 2E; ANOVA; $p < 0.05$, $n = 9–10$). In the absence of ethanol, the *fmn*'s baseline activities were similar in all social conditions and sexes (two-way ANOVA: social setting, $F_{1,38} = 0.01$, $p = 0.92$; sex effect, $F_{1,38} = 0.03$, $p = 0.87$; social setting × sex interaction, $F_{1,38} = 0.08$, $p = 0.776$). Compared with *CS*, however, the *fmn*'s response to ethanol was substantially augmented and prolonged in a social setting but similar in a single setting (Figure 2F: female two-way ANOVA: genotype, $F_{3,39} = 61.03$, $p < 0.0001$; social setting, $F_{3,38} = 98.42$, $p < 0.0001$; genotype × social setting, $F_{3,38} = 58.70$, $p < 0.0001$; male two-way ANOVA: genotype, $F_{3,39} = 42.1$, $p < 0.0001$; social setting, $F_{3,38} = 91.38$, $p < 0.0005$; genotype × social setting, $F_{3,38} = 50.49$, $p < 0.0001$. $n = 9–11$). This suggests that both hyper dopamine activity and social enrichment interact for the augmented hyperlocomotor activity under the influence of ethanol.

### 3.3 | The social context-associated ethanol-induced hyperactivity requires the D1 dopamine receptor dDA1/Dop1R1

Dopamine is a key neurotransmitter for the ethanol-induced hyperlocomotor activity.[24,25] We first surveyed all dopamine receptors regarding their contributions to the ethanol-induced hyperactivity. To do this, we administered ethanol to individual dopamine receptor mutants; *dumb*$^1$ and *dumb*$^4$ defective in dDA1/Dop1R1 (D1), *d2r* defective in dD2R/Dop2R (D2), *damb* defective in DAMB/Dop1R2 (D5), and *der* defective in dopamine/ecdysone receptor (DopEcR).[26,28–30] All dopamine receptor mutants, regardless of sex, showed ethanol-induced hyperactivity in both social settings (Figure 3A–D, $n = 9–11$). These data suggest that deficiency in a single dopamine receptor does not affect hyperlocomotor response to ethanol.

We next investigated which dopamine receptor is important for the social context-sensitive ethanol responses. When we compared the top speeds of the dopamine receptor mutants exposed to ethanol in a single versus social setting, the *d2r*, *damb*, and *der* mutant females showed the enhanced hyperactivity in a social setting whereas *dumb*$^1$ and *dumb*$^4$ did not show significant difference between single and social settings (Figure 3E, $n = 10–11$). In males, on the other hand, *d2r* and *der* mutants showed the enhanced hyperactivity in a social setting while *dumb* and *damb* mutants did not show significant differences (Figure 3F, $n = 9–11$). In the absence of ethanol, the social environment did not affect the baseline activities of all the dopamine receptor mutants while there were differences in basal activities among different genotypes (female two-way ANOVA: social setting, $F_{1,122} = 0.03$, $p = 0.874$; genotype, $F_{5,122} = 6.74$, $p < 0.0001$; genotype × social setting, $F_{5,122} = 58.70$, $p = 0.999$; male two-way ANOVA: social setting, $F_{1,124} = 0.616$, $p = 0.874$; genotype, $F_{5,124} = 6.73$, $p < 0.0001$; genotype × social setting, $F_{5,124} = 58.70$, $p = 1.000$). Together, these data indicate that the D1 receptor dDA1/Dop1R1 is important for the social environment-sensitive ethanol-induced hyperlocomotor activity in both sexes while DAMB/Dop1R2 is also involved in this process only in males.

### 3.4 | The dDA1/Dop1R1 is critical for the *fmn*'s social context-sensitive ethanol-induced hyperactivity

Because dDA1/Dop1R1 is a key receptor for the social context effect, we asked whether dDA1/Dop1R1 is also responsible for the augmented hyperactivity of the *fmn* and social context interaction. To address this, we examined the double mutant *fmn;dumb*$^4$ defective in both DAT and dDA1//Dop1R1 upon exposure to ethanol in a social setting. The double mutant showed the ethanol response comparable with those of *CS* and *dumb*$^4$, suggesting that dDA1/Dop1R1 deficiency substantially dampened the *fmn*'s ethanol-induced hyperactivity (Figure 4A: group female ANOVA: $F_{3,52} = 14.05$, $p < 0.0001$; group male ANOVA: $F_{3,52} = 24.17$, $p < 0.0001$; $n = 14$). The singly exposed flies of all genotypes, on the other hand, showed similar locomotor responses and top speeds (Figure 4B: single female ANOVA: $F_{3,52} = 0.28$, $p = 0.837$; single male ANOVA: $F_{3,52} = 1.51$, $p = 0.226$, $n = 14$). The baseline activities of all genotypes were not impacted by the social setting (female two-way ANOVA: social setting, $F_{1,111} = 0.001$, $p = 0.949$; genotype, $F_{3,111} = 140.63$, $p < 0.0001$; genotype × social setting, $F_{3,111} = 0.03$, $p = 0.992$; male two-way ANOVA: social setting, $F_{1,111} = 0.12$, $p = 0.735$; genotype, $F_{3,111} = 156.72$, $p < 0.0001$; genotype × social setting, $F_{3,111} = 0.001$, $p = 1.000$). These data indicate that dDA1/Dop1R1 is a key receptor mediating the *fmn* and social environment interaction for the locomotor response to ethanol.

## 4 | DISCUSSION

In this study directed at investigating the effect of a social setting on ethanol response, we found that a social setting, compared with a solitary setting, promotes the ethanol-induced hyperlocomotor activity and hyper dopamine further augments it via the D1 receptor dDA1/Dop1R1. The hyperlocomotor activity in animal models represents a stimulant or euphoric effect of ethanol.[15] Our findings thus support

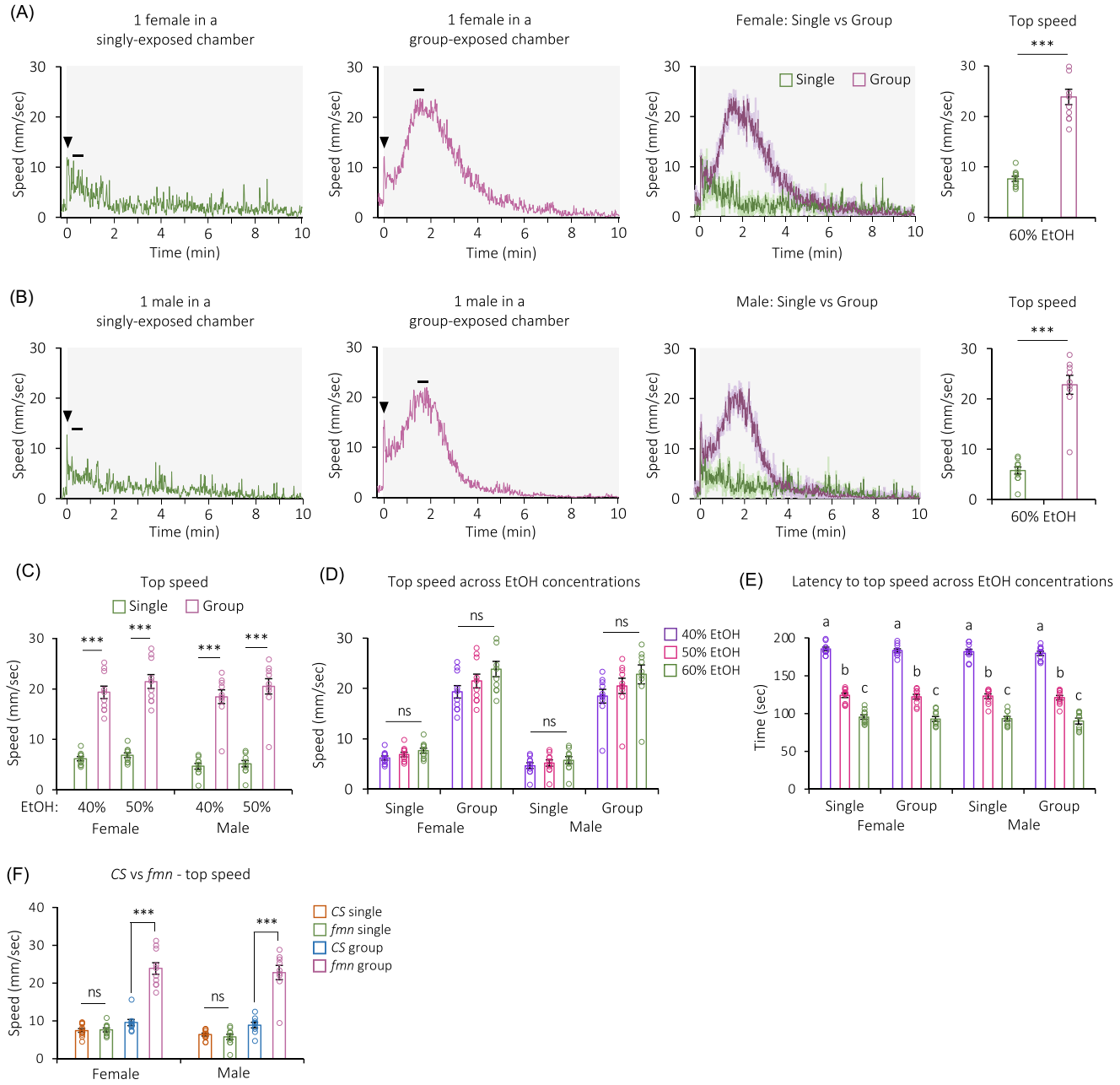


**FIGURE 2** Concurrent social enrichment and increased dopamine signalling amplifies ethanol-induced hyperactivity. The *fmn* flies with the homozygous mutation in DAT (group-exposed in magenta and singly exposed in green) and *CS* (group-exposed in blue; singly exposed in orange) were exposed to ethanol. Shown from left to right are a single fly's walking speed trace in a singly exposed chamber, a single fly trace in a group-exposed chamber, the average of multiple chambers per social setting with SEM in shaded colour, and top speeds (rightmost panel) of females (A) and males (B) exposed to 60% ethanol. In both sexes, the group-exposed *fmn* displayed highly augmented locomotor responses to ethanol compared with the singly exposed *fmn* (***$p < 0.0001$; $n = 9–10$). (C) the top speeds of *fmn* females and males to either 40% or 50% ethanol in different social settings were compared. In both 40% and 50% ethanol concentrations, the group-exposed *fmn* displayed significantly higher top speeds compared with singly exposed *fmn* (***$p < 0.0001$; $n = 10$). (D) the *fmn*'s top speeds during exposure to various ethanol concentrations. The *fmn*'s top speeds were not different in all social conditions and sexes tested (ns, $p > 0.05$; $n = 9–10$). (E) the *fmn*'s latencies to the ethanol-induced top speeds. The *fmn* latencies to top speeds decreased with higher ethanol concentrations (different letters denote significant differences; $n = 9–10$). (F) the top speeds of *CS* and *fmn* in both social settings were compared. In both sexes, the group-exposed *fmn* exhibited greater locomotor responses to ethanol compared with the singly exposed *fmn* and singly and group-exposed *CS* (ns, $p > 0.05$; ***$p < 0.0001$; $n = 9–10$)

the previous studies on human subjects reporting that social drinking elevates euphoria and ethanol's stimulant effect.[3,33] The effects of social drinking have been studied in animal models, but they focused on ethanol consumption or preferences. This study, to our knowledge, is the first study in an animal model demonstrating the positive impact of social context on the ethanol's stimulant effect. In the Wolf et al.

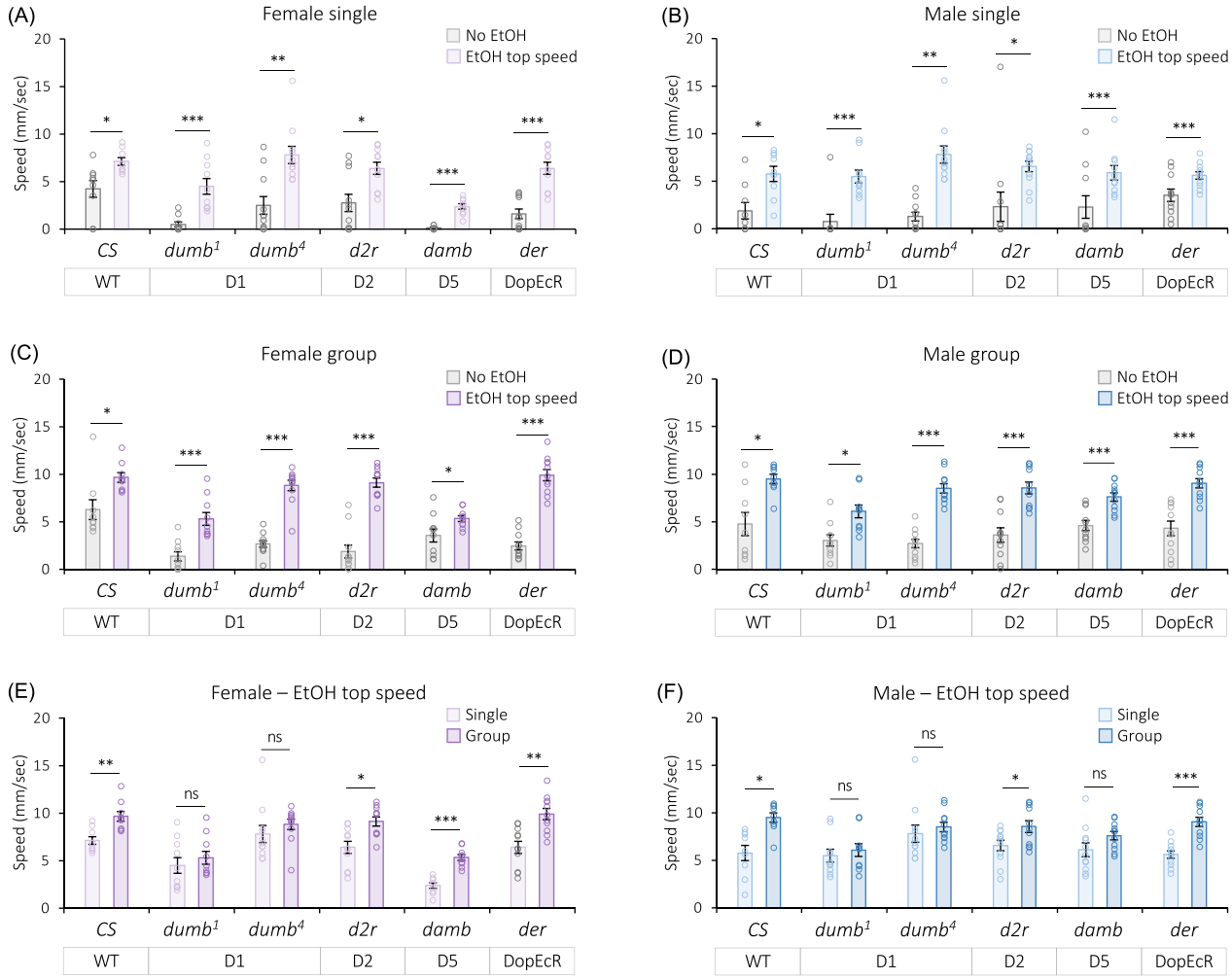


**FIGURE 3** Dopamine receptors involved in the social context-dependent ethanol-induced hyperactivity. The mutants *dumb*$^1$ and *dumb*$^4$ defective in D1 receptor, *d2r* defective in D2 receptor, *damb* defective in D5 receptor, and *der* defective in DA/ecdysone receptor were exposed to ethanol in a single or group setting and their top speeds during exposure and 30 s prior to exposure (denoted as "no EtOH"; EtOH, ethanol) are shown. (A)–(D) the movement speeds in the absence of ethanol (single: light grey, group: dark grey) and the tops speeds during ethanol exposure of females (single: light purple, group: dark purple) and males (single: light blue, group: dark blue) were compared. (A),(B) in single exposure, both sexes displayed significantly higher top speeds during ethanol exposure compared with the absence of ethanol for all mutants as well as *CS* (*$p < 0.05$, **$p < 0.001$; ***$p < 0.0001$; $n = 9$–11). (C),(D) in group exposure, both sexes displayed significantly higher top speeds during ethanol exposure compared with no ethanol for all mutants as well as *CS* (*$p < 0.05$, ***$p < 0.0001$; $n = 9$–11). (E),(F) the top speeds of single- and group-exposed dopamine receptor mutant flies during ethanol exposure were compared. (E) in females, *dumb*$^1$ and *dumb*$^4$ flies did not show any significant difference between the singly exposed and group-exposed flies while *CS*, *d2r*, *damb*, and *der* group-exposed flies displayed significantly higher locomotor responses to ethanol compared with singly exposed flies (ns, not significant, $p > 0.05$; *$p < 0.05$; **$p < 0.001$; ***$p < 0.0001$; $n = 9$–11). (F) in males, *dumb*$^1$, *dumb*$^4$, and *damb* flies did not show any significant difference between the singly exposed and group-exposed flies whereas *CS*, *d2r*, and *der* group-exposed flies displayed higher locomotor responses to ethanol compared with singly exposed flies (ns, $p > 0.05$; *$p < 0.05$; ***$p < 0.0001$; $n = 9$–11)

study,[14] the authors noted that "the average behavior of multiple individual flies does not differ from that of a population of flies" when the activity pattern such as the percent time spent on fast locomotion, bout length, and bout frequency under the ethanol influence was measured. This indicates that the top speed likely representing a peak euphoric response, but not the activity pattern, is sensitive to social environment.

Our study also shows the synergistic interaction of social context and hyper dopamine on the ethanol's stimulant effect. This is in line with the studies on human subjects reporting the impact of social drinking on euphoria and ethanol's stimulant effect being significantly greater in the individuals with extraversion personal trait[34] that is linked to high levels of dopaminergic activity.[35,36] The dopamine transporter mutant *fmn* was shown to have reduced, as opposed to increased, locomotor response to ethanol,[25] which is contrary to our study. The *fmn* mutant flies in the Kong et al. study are in the *white* mutant genetic background whereas *fmn* mutants in our study are in the wild-type *CS* background with the wild-type *white* allele. The *white*

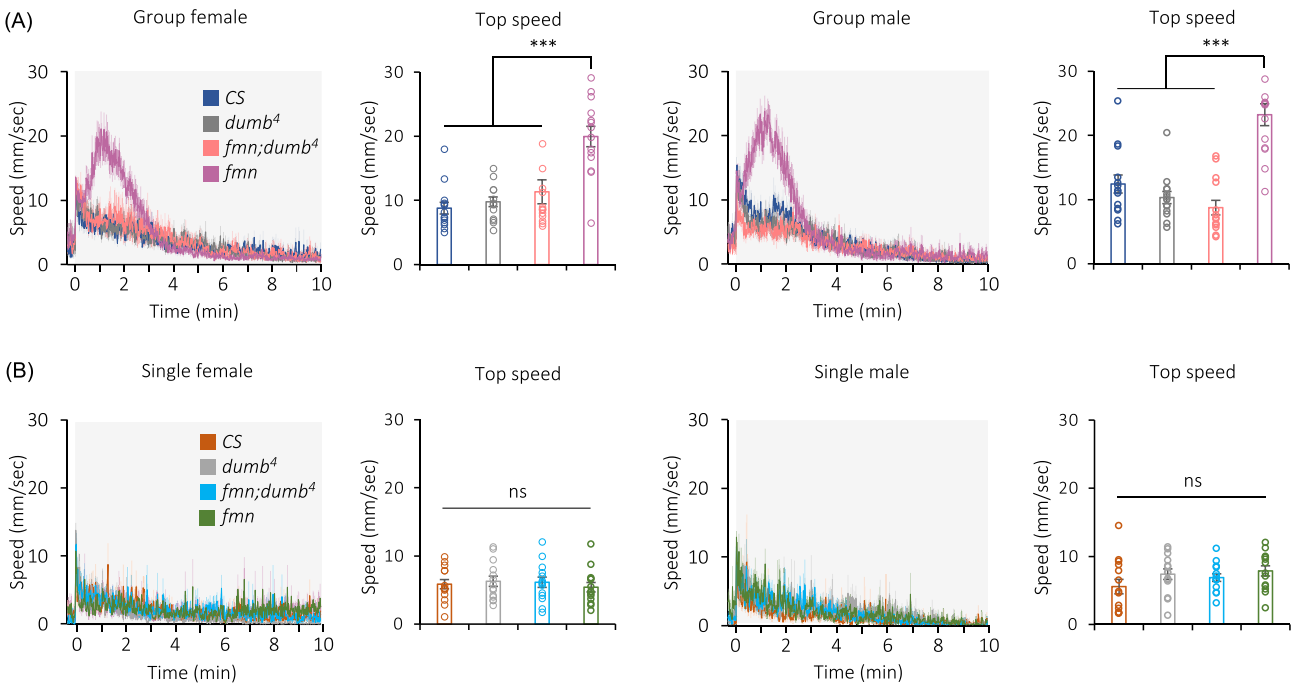


**FIGURE 4** The D1 receptor dDA1/Dop1R1 is critical for the social context-sensitive ethanol responses. (A) the double mutant *fmn;dumb*$^4$ defective in both DAT and dDA1/Dop1R1 (pink), *CS* (blue), *dumb*$^4$ (dark grey), and *fmn* (magenta) were exposed to ethanol in a group setting, and their locomotor responses were compared. In both sexes, the group-exposed *CS*, *dumb*$^4$, and *fmn;dumb*$^4$ displayed significantly reduced locomotor responses to ethanol compared with *fmn* (***$p < 0.0001$; $n = 14$). (B) the *fmn;dumb*$^4$ (light blue), *CS* (orange), *dumb*$^4$ (light grey), and *fmn* (green) were exposed to ethanol in a single setting. In both sexes, all genotypes displayed similar locomotor responses (ns, $p > 0.05$; $n = 14$)

gene codes for a subunit of ATP-binding cassette transporter important for eye pigmentation[37] and is also expressed beyond eye pigment cells.[38,39] The *white* mutants are defective in courtship,[40] copulation,[41] locomotor behaviour,[42] ethanol-induced sedation,[16] and learning and memory[43,44] among others. The discrepancy in the ethanol response is likely contributed by the genetic background. Notably, the individuals experiencing greater euphoria from drinking have an increased risk of alcohol use disorder,[6] and consistently, the dopamine transporter polymorphism is associated with alcohol use disorder.[45–48] Thus, our finding and follow-up study on mechanism may provide key insight into how social context contributes to alcohol use disorder.

Dopamine neurotransmission is important for the stimulant effect of ethanol as well as social interaction in human subjects,[35,36,49] rodents,[50–52] and flies.[52–55] Alcohol drinking can occur in socially isolated or enriched environment, but how solitary versus social drinking impacts dopamine signalling is unknown. We found that the flies deficient in the D1 dopamine receptor dDA1/Dop1R1 are responsive to the ethanol's stimulant effect in both solitary and social settings but insensitive to the effect of social setting, pinpointing D1 receptor as a major receptor mediating the social context-sensitive stimulant effect of ethanol. We further identify that D1 receptor function is required for the synergistic interaction of social context and hyper dopamine on the ethanol's stimulant effect. D1 receptor has been shown important for social interactions in rodents[50,51] and courtship motivation of male flies.[29] This study underscores the critical role of D1 receptor in convergent processing of multiple salient information such as ethanol and social recognition. The sexually dimorphic function of the D5 receptor DAMB/Dop1R2 is notable: DAMB/Dop1R2 deficiency is indispensable in female flies but abolishes the social context effect on ethanol response in male flies. D5 receptor function has not been linked to ethanol response and social interaction. The follow-up study on the mechanism by which DAMB/Dop1R2 mediates the social context effect will surely help narrow this knowledge gap. Taken together, this study provides a useful framework to uncover the mechanism by which dopamine signalling via D1 and D5 mediates the impact of social context and its synergism with hyper dopamine on the ethanol's stimulant effect, which is relevant to alcohol use disorder.

In this study we focused on the impact of social context on the locomotor-activating effect of ethanol. Ethanol also induces loss of movement control, hypoactivity, and sedation. It will be informative to explore whether social environment influences these ethanol effects in a follow-up study. We noted that the behavioural patterns of dopamine receptor mutants under the influence of ethanol as well as in the absence of ethanol are heterogeneous and deserve further characterization in a follow-up study.

## AUTHOR CONTRIBUTIONS

P. R. S. and K. A. H. designed the experiments; D. M. G., B. H. G., and P. R. S. performed and analysed all experiments; D. M. G., B. H. G., and P. R. S. prepared all figures. D. M. G. and K. A. H. wrote the manuscript with input from B. H. G. and P. R. S.

## ACKNOWLEDGMENTS

This work was supported by the Orville Edward Egbert, M.D. Endowment fund, NIH NIGMS 1R16GM145548 and NIH NIMHD 3U54MD007592-29S5 grants. We are grateful for the Bloomington Stock Center and the Exelixis Collection at Harvard Medical School for fly lines. We appreciate the past and current lab members for their discussion and support.

## CONFLICT OF INTEREST STATEMENT

All authors declare no competing financial interests.

## DATA AVAILABILITY STATEMENT

All the raw data and materials used in the study are available upon request from the corresponding author.

## ORCID

*Paul Rafael Sabandal* https://orcid.org/0009-0006-6845-5978
*Kyung-An Han* https://orcid.org/0000-0001-7972-1764

## REFERENCES

1. Abbey A, Smith MJ, Scott RO. The relationship between reasons for drinking alcohol and alcohol consumption: an interactional approach. *Addict Behav*. 1993;18(6):659-670. doi:10.1016/0306-4603(93)90019-6
2. Young-Wolff KC, Enoch MA, Prescott CA. The influence of gene-environment interactions on alcohol consumption and alcohol use disorders: a comprehensive review. *Clin Psychol Rev*. 2011;31(5):800-816. doi:10.1016/j.cpr.2011.03.005
3. Doty P, de Wit H. Effect of setting on the reinforcing and subjective effects of ethanol in social drinkers. *Psychopharmacology (Berl)*. 1995;118(1):19-27. doi:10.1007/BF02245245
4. Ju YJ, Kim W, Oh SS, Park EC. Solitary drinking and the risk of depressive symptoms and suicidal ideation in college students: findings from a nationwide survey in Korea. *J Affect Disord*. 2019;257:710-715. doi:10.1016/j.jad.2019.07.080
5. Skrzynski C, Creswell KG, Bachrach RL, Chung T. Social discomfort moderates the relationship between drinking in response to negative affect and solitary drinking in underage drinkers. *Addict Behav*. 2018;78:124-130. doi:10.1016/j.addbeh.2017.11.009
6. Creswell KG. Drinking together and drinking alone: a social-contextual framework for examining risk for alcohol use disorder. *Curr Dir Psychol Sci*. 2021;30(1):19-25. doi:10.1177/0963721420969406
7. Varlinskaya EI, Spear LP, Spear NE. Acute effects of ethanol on behavior of adolescent rats: role of social context. *Alcohol Clin Exp Res*. 2001;25(3):377-385. doi:10.1111/j.1530-0277.2001.tb02224.x
8. Varlinskaya EI, Spear LP. Acute effects of ethanol on social behavior of adolescent and adult rats: role of familiarity of the test situation. *Alcohol Clin Exp Res*. 2002;26(10):1502-1511. doi:10.1111/j.1530-0277.2002.tb02449.x
9. Varlinskaya EI, Truxell EM, Spear LP. Ethanol intake under social circumstances or alone in Sprague-Dawley rats: impact of age, sex, social activity, and social anxiety-like behavior. *Alcohol Clin Exp Res*. 2015;39(1):117-125. doi:10.1111/acer.12604
10. Fulenwider HD, Robins MT, Caruso MA, Ryabinin AE. Social housing leads to increased ethanol intake in male mice housed in environmentally enriched cages. *Front Behav Neurosci*. 2021;15:695409. doi:10.3389/fnbeh.2021.695409
11. Strickland JC, Smith MA. Animal models of social contact and drug self-administration. *Pharmacol Biochem Behav*. 2015;136:47-54. doi:10.1016/j.pbb.2015.06.013
12. Devineni AV, Heberlein U. The evolution of Drosophila melanogaster as a model for alcohol research. *Annu Rev Neurosci*. 2013;36(1):121-138. doi:10.1146/annurev-neuro-062012-170256
13. W McKechnie S, Morgan P. Alcohol dehydrogenase polymorphism of *Drosophila melanogaster*: aspects of alcohol and temperature variation in the larval environment. *Aust J Biol Sci*. 1982;35(1):85-94. doi:10.1071/BI9820085
14. Wolf FW, Rodan AR, Tsai LT, Heberlein U. High-resolution analysis of ethanol-induced locomotor stimulation in drosophila. *J Neurosci*. 2002;22(24):11035-11044. doi:10.1523/JNEUROSCI.22-24-11035.2002
15. Phillips TJ, Shent EH. Neurochemical bases of locomotion and ethanol stimulant effects. *Int Rev Neurobiol*. 1996;39:243-282. doi:10.1016/S0074-7742(08)60669-8
16. Lee HG, Kim YC, Dunning JS, Han KA. Recurring ethanol exposure induces disinhibited courtship in drosophila. *PLoS ONE*. 2008;3(1):e1391. doi:10.1371/journal.pone.0001391
17. Shohat-Ophir G, Kaun KR, Azanchi R, Mohammed H, Heberlein U. Sexual deprivation increases ethanol intake in drosophila. *Science*. 2012;335(6074):1351-1355. doi:10.1126/science.1215932
18. Eddison M, Guarnieri DJ, Cheng L, et al. Arouser reveals a role for synapse number in the regulation of ethanol sensitivity. *Neuron*. 2011;70(5):979-990. doi:10.1016/j.neuron.2011.03.030
19. Boileau I, Assaad JM, Pihl RO, et al. Alcohol promotes dopamine release in the human nucleus accumbens. *Synapse*. 2003;49(4):226-231. doi:10.1002/syn.10226
20. Imperato A, Di Chiara G. Preferential stimulation of dopamine release in the nucleus accumbens of freely moving rats by ethanol. *J Pharmacol Exp Ther*. 1986;239(1):219-228.
21. Di Chiara G. Alcohol and Dopamine. In: *Alcohol health and research world*. Vol.21; 1997:108-114.
22. Carlsson A, Lindqvist M. Effect of ethanol on the hydroxylation of tyrosine and tryptophan in rat brain in vivo. *J Pharm Pharmacol*. 1973;25(6):437-440. doi:10.1111/j.2042-7158.1973.tb09129.x
23. Lyness WH, Smith FL. Influence of dopaminergic and serotonergic neurons on intravenous ethanol self-administration in the rat. *Pharmacol Biochem Behav*. 1992;42(1):187-192. doi:10.1016/0091-3057(92)90465-R
24. Bainton RJ, Tsai LT, Singh CM, Moore MS, Neckameyer WS, Heberlein U. Dopamine modulates acute responses to cocaine, nicotine and ethanol in drosophila. *Curr Biol*. 2000;10(4):187-194. doi:10.1016/S0960-9822(00)00336-5
25. Kong EC, Woo K, Li H, et al. A pair of dopamine neurons target the D1-like dopamine receptor DopR in the central complex to promote ethanol-stimulated locomotion in drosophila. *PLoS ONE*. 2010;5(4):e9954. doi:10.1371/journal.pone.0009954
26. Aranda GP, Hinojos SJ, Sabandal PR, Evans PD, Han KA. Behavioral sensitization to the disinhibition effect of ethanol requires the dopamine/ecdysone receptor in drosophila. *Front Syst Neurosci*. 2017;11:56. doi:10.3389/fnsys.2017.00056
27. Kume K, Kume S, Park SK, Hirsh J, Jackson FR. Dopamine is a regulator of arousal in the fruit fly. *J Neurosci*. 2005;25(32):7377-7384. doi:10.1523/JNEUROSCI.2048-05.2005
28. Kim YC, Lee HG, Han KA. D1 dopamine receptor dDA1 is required in the mushroom body neurons for aversive and appetitive learning in drosophila. *J Neurosci*. 2007;27(29):7640-7647. doi:10.1523/JNEUROSCI.1167-07.2007
29. Lim J, Fernandez AI, Hinojos SJ, et al. The mushroom body D1 dopamine receptor controls innate courtship drive. *Genes Brain Behav*. 2018;17(2):158-167. doi:10.1111/gbb.12425
30. Cassar M, Issa AR, Riemensperger T, et al. A dopamine receptor contributes to paraquat-induced neurotoxicity in drosophila. *Hum Mol Genet*. 2015;24(1):197-212. doi:10.1093/hmg/ddu430

31. Himmelreich S, Masuho I, Berry JA, et al. Dopamine receptor DAMB signals via Gq to mediate forgetting in drosophila. *Cell Rep*. 2017; 21(8):2074-2081. doi:10.1016/j.celrep.2017.10.108
32. Makos MA, Kim YC, Han KA, Heien ML, Ewing AG. In vivo electrochemical measurements of exogenously applied dopamine in Drosophila melanogaster. *Anal Chem*. 2009;81(5):1848-1854. doi:10.1021/ac802297b
33. Kirkpatrick MG, de Wit H. In the company of others: social factors alter acute alcohol effects. *Psychopharmacology (Berl)*. 2013;230(2): 215-226. doi:10.1007/s00213-013-3147-0
34. Fairbairn CE, Sayette MA, Wright AG, Levine JM, Cohn JF, Creswell KG. Extraversion and the rewarding effects of alcohol in a social context. *J Abnorm Psychol*. 2015;124(3):660-673. doi:10.1037/abn0000024
35. Wacker J, Smillie LD. Trait extraversion and dopamine function. *Social Pers Psychol Compass*. 2015;9(6):225-238. doi:10.1111/spc3.12175
36. Humińska-Lisowska K, Chmielowiec K, Strońska-Pluta A, et al. Epigenetic analysis of the dopamine transporter gene. *Int J Mol Sci*. 2023; 24(10):8931. doi:10.3390/ijms24108931
37. Mackenzie SM, Brooker MR, Gill TR, Cox GB, Howells AJ, Ewart GD. Mutations in the white gene of Drosophila melanogaster affecting ABC transporters that determine eye colouration. *Biochim Biophys Acta*. 1999;1419(2):173-185. doi:10.1016/S0005-2736(99)00064-4
38. Evans JM, Day JP, Cabrero P, Dow JA, Davies SA. A new role for a classical gene: White transports cyclic GMP. *J Exp Biol*. 2008;211(Pt 6):890-899. doi:10.1242/jeb.014837
39. Li H, Janssens J, De Waegeneer M, et al. Fly cell atlas: a single-nucleus transcriptomic atlas of the adult fruit fly. *Science*. 2022; 375(6584):eabk2432. doi:10.1126/science.abk2432
40. Krstic D, Boll W, Noll M. Influence of the White locus on the courtship behavior of drosophila males. *PLoS ONE*. 2013;8(10):e77904. doi:10.1371/journal.pone.0077904
41. Xiao C, Qiu S, Robertson RM. The white gene controls copulation success in Drosophila melanogaster. *Sci Rep*. 2017;7(1):7712. doi:10.1038/s41598-017-08155-y
42. Liu L, Davis RL, Roman G. Exploratory activity in drosophila requires the kurtz nonvisual arrestin. *Genetics*. 2007;175(3):1197-1212. doi:10.1534/genetics.106.068411
43. Sitaraman D, Zars M, Laferriere H, et al. Serotonin is necessary for place memory in drosophila. *Proc Natl Acad Sci U S A*. 2008;105(14): 5579-5584. doi:10.1073/pnas.0710168105
44. Myers JL, Porter M, Narwold N, Bhat K, Dauwalder B, Roman G. Mutants of the white ABCG transporter in Drosophila melanogaster have deficient olfactory learning and cholesterol homeostasis. *Int J Mol Sci*. 2021;22(23):12967. doi:10.3390/ijms222312967
45. Muramatsu T, Higuchi S. Dopamine transporter gene polymorphism and alcoholism. *Biochem Biophys Res Commun*. 1995;211(1):28-32. doi:10.1006/bbrc.1995.1773
46. Sander T, Harms H, Podschus J, et al. Allelic association of a dopamine transporter gene polymorphism in alcohol dependence with withdrawal seizures or delirium. *Biol Psychiatry*. 1997;41(3):299-304. doi:10.1016/S0006-3223(96)00044-3
47. Schmidt LG, Harms H, Kuhn S, Rommelspacher H, Sander T. Modification of alcohol withdrawal by the A9 allele of the dopamine transporter gene. *Am J Psychiatry*. 1998;155(4):474-478. doi:10.1176/ajp.155.4.474
48. Ueno S, Nakamura M, Mikami M, et al. Identification of a novel polymorphism of the human dopamine transporter (DAT1) gene and the significant association with alcoholism. *Mol Psychiatry*. 1999;4(6):552-557. doi:10.1038/sj.mp.4000562
49. Smillie LD, Wacker J. Dopaminergic foundations of personality and individual differences. *Front Hum Neurosci*. 2014;8:874.
50. Homberg JR, Olivier JD, VandenBroeke M, et al. The role of the dopamine D1 receptor in social cognition: studies using a novel genetic rat model. *Dis Model Mech*. 2016;9(10):1147-1158. doi:10.1242/dmm.024752
51. Matta R, Tiessen AN, Choleris E. The role of dorsal hippocampal dopamine D1-type receptors in social learning, social interactions, and food intake in male and female mice. *Neuropsychopharmacology*. 2017;42(12):2344-2353. doi:10.1038/npp.2017.43
52. Ike KGO, Lamers SJC, Kaim S, et al. The human neuropsychiatric risk gene Drd2 is necessary for social functioning across evolutionary distant species. *Mol Psychiatry*. 2024;29(2):518-528. doi:10.1038/s41380-023-02345-z
53. Fernandez RW, Akinleye AA, Nurilov M, et al. Modulation of social space by dopamine in. *Biol Lett*. 2017;13(8):20170369. doi:10.1098/rsbl.2017.0369
54. Xie T, Ho MCW, Liu Q, et al. A genetic toolkit for dissecting dopamine circuit function in drosophila. *Cell Rep*. 2018;23(2):652-665. doi:10.1016/j.celrep.2018.03.068
55. Campbell NG, Shekar A, Aguilar JI, et al. Structural, functional, and behavioral insights of dopamine dysfunction revealed by a deletion inSLC6A3. *Proc Natl Acad Sci U S A*. 2019;116(9):3853-3862. doi:10.1073/pnas.1816247116

**How to cite this article:** Murillo Gonzalez DJ, Hernandez Granados BA, Sabandal PR, Han K-A. Social setting interacts with hyper dopamine to boost the stimulant effect of ethanol. *Addiction Biology*. 2024;29(6):e13420. doi:10.1111/adb.13420