Title:

Noradrenergic and Pavlovian underpinnings of compulsive versus adaptive coping strategies

Authors

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Authors contribution:

DB and ABR designed the experiments. ABR, AHM, ST, BN, CY and MP carried out the beahvioural experiments. ABR and MF carried out the molecular biology experiments. CC, SJ and DB analysed and interpreted the data and wrote the manuscript.

Abstract

Loss of control over coping strategies can result in the development of impulsive/compulsive spectrum disorders (ICSDs) such as Obsessive-Compulsive Disorder or trichotillomania. Rats, like humans, show individual differences in their tendency to engage in and maintain control over coping behaviours. While most rats exposed to a schedule-induced polydipsia (SIP) procedure develop a controlled coping response, namely polydipsic drinking, some engage in excessive, compulsive drinking, or hyperdipsia. The development of hyperdipsia, to which highly impulsive rats are particularly vulnerable, depends on noradrenergic mechanisms as it is prevented by the noradrenaline reuptake inhibitor, atomoxetine. However, whether noradrenergic mechanisms also underlie the maintenance of well-established compulsive coping or if other traits confer vulnerability to its development, are unknown. In two longitudinal studies in male Sprague Dawley rats, we investigated whether well-established hyperdipsia is influenced by atomoxetine and if its development is predicted by the ICSD-relevant sign-tracking trait. Sign-tracking predicted faster acquisition of adjunctive drinking but not the transition to hyperdipsia, which goal-tracking protected against. Surprisingly, chronic atomoxetine exacerbated well-established hyperdipsia. Post-mortem qPCR assays revealed that the exacerbation of compulsive coping by atomoxetine was associated with increased mRNA levels of the markers of cellular activity and plasticity c-fos and Zif268 across the dorsal striatum. Atomoxetine also altered the hyperdipsia-specific transcriptomic landscape of the nucleus accumbens shell and the pattern of c-fos and Zif268 expression in the amygdala-accumbens system. These results provide new insights into the biobehavioural basis of compulsive behaviours, revealing a differential noradrenergic control of the development and the expression of compulsive coping.

Introduction

Stress is inevitable, and the ability to adaptively regulate and cope with negative emotions is paramount for resilience against stressors (1–4). However, some coping strategies become excessive and rigid, characteristic of psychiatric disorders such as mood/anxiety disorders (3,5,6), impulsive/compulsive spectrum disorders (ICSDs) (4,7–11), and substance use disorders (SUDs) (12–14).

Adjunctive behaviours are a form of displacement behaviour expressed by many species as a means of coping with stress (15–19). An example of adjunctive coping is polydipsic drinking under a schedule-induced polydipsia (SIP) procedure, wherein food-deprived rats drink supra-homeostatic volumes of water in response to the stress induced by predictable, intermittent food delivery (20,21). Polydipsic drinking is anxiolytic, as it reduces plasma corticosterone levels (21–24) and anxiety-like behaviour (25,26), which are both increased by periodic food delivery in food-restricted individuals (26–28). While the majority of rats maintain a stable, controlled level of polydipsic drinking, some lose control over their coping response and develop maladaptive, excessive SIP drinking (24–26,28–31) referred to as *hyper*dipsia. Hyperdipsia is compulsive in nature as it results in expending energy, whilst already in a caloric deficit, to consume, warm, and excrete non-regulatory volumes of water (24–26,32–34). While the acquisition of polydipsic coping responses depends on the mesolimbic dopaminergic system (35–37), well-established compulsive hyperdipsia is controlled by the dopamine-dependent anterior dorsolateral striatum (aDLS) habit system (25) – a shift in the neural locus of control over behaviour analogous to that which accompanies the emergence of compulsive drug-seeking habits (38–42) and OCD-like repetitive behaviours (43).

The biobehavioural factors contributing to population-level heterogeneity in coping responses, such as adjunctive water drinking, and the unique vulnerability to develop compulsive manifestations of these responses in select individuals, are not yet well understood. Nevertheless, impaired interoception (44,45) has been suggested to play a determinant role in the development of compulsive coping behaviours, including hyperdipsia (26,46), possibly by promoting greater reliance on exteroceptive contextual stimuli versus interoceptive affective and homeostatic cues to guide behaviour, as supported by the role of interoceptive acuity in *resilience* to stress-related psychopathology (47–50). The anterior insula, a neural locus of interoception (51,52), is critical for the development and expression of several compulsive behaviours, including hyperdipsia (26). Interestingly, it is also involved in motor impulsivity (26) and sign-tracking (53) (the propensity to attribute incentive value to, and thus approach, reward-predictive conditioned stimuli (CSs)) (54–56). Sign-trackers may also readily form Pavlovian associations with interoceptive stimuli, so that their behaviour may be more amenable to the motivational influence of internal cues (57). Accordingly, sign-trackers may more readily associate the internal state elicited by food deprivation with the anxiolytic effects of polydipsic drinking in the SIP context, a Pavlovian motivational process that would facilitate the development of this coping response.

At the neurochemical level, catecholaminergic mechanisms in the nucleus accumbens (NAc) underpin both the expression of sign-tracking (58–61) and motor impulsivity (62–64), and the development of adjunctive coping under a SIP procedure (35–37). Yet, the neurochemical mechanisms underlying hyperdipsia appear to be distinct from those of adaptive, controlled coping (24,29,31,65,66). The development of hyperdipsia, to which highly impulsive rats are particularly vulnerable (29,31,66), depends on noradrenergic mechanisms as it is prevented by the noradrenaline reuptake inhibitor, atomoxetine (29), a first-line medication for ADHD that also decreases impulsivity in these individuals. In contrast, atomoxetine has no effect on the development of non-compulsive, adaptive, polydipsic drinking (29). This differential response to

pharmacological manipulations of the noradrenergic system in vulnerable and resilient rats resonates with the higher extracellular noradrenaline concentration observed in hyperdipsia-vulnerable high impulsive rats compared to their low impulsive counterparts (66) in the nucleus accumbens (NAc), the shell of which (NAcS) is the striatal region in which atomoxetine acts to ameliorate impulsivity (62). Further, over the course of the development of hyperdipsia, compulsive rats also develop abnormalities in the level of expression of the plasticity marker Arc in a neuronal ensemble of the noradrenergic locus coeruleus (LC) (24). These transcriptomic alterations in the LC persist long after hyperdipsia has become reliant on the aDLS-dopamine dependent habit system (25), yet the LC projects exclusively to the NAcS in the striatum (24,67,68). Thus, while aberrations in the noradrenaline system confer vulnerability to develop compulsive coping, it remains unclear how they are behaviourally manifested besides impulsivity, i.e., if they contribute to sign tracking, or how noradrenergic mechanisms orchestrate the maintenance of well-established, habitual aDLS-dopamine dependent hyperdipsia.

Thus, in two longitudinal studies in male Sprague Dawley rats, we investigated (i) if sign-tracking predicts an increased tendency to develop hyperdipsia, and (ii) whether well-established hyperdipsia or sign-tracking is influenced by atomoxetine. Using post-mortem molecular biology assays, we further characterized the influence of atomoxetine on (iii) the functional signature of hyperdipsia within the corticostriatal circuitry and (iv) compulsion-specific expression patterns of candidate genes in the NAcS.

Materials and Methods

Subjects

Forty-eight and sixty adult male Sprague-Dawley rats were used for Experiment 1 and 2, respectively. Rats were 10 weeks of age at the start of the experiment, and were food restricted to 85-90% or 80-83% of theoretical free-feeding weight prior to autoshaping or SIP training, respectively. Water was freely available in the homecage. All procedures were conducted under the project license 70/8072 held by David Belin in accordance with the requirements of the UK Animals (Scientific Procedures) Act 1986, amendment 2012. Two animals in Experiment 2 were excluded due to either excessive weight loss, despite intensive supportive care, or persistent emptying of the entire 300mL water bottle.

Experimental design

Experiment 1 aimed to examine the relationship between sign-tracking and the propensity to develop polydipsic drinking or its compulsive manifestation, hyperdipsia, by first screening rats (n=48) for trait sign-tracking and goal-tracking in an autoshaping task (as described below), followed by training under a SIP procedure (**Figure 1A**). Experiment 1 also enabled us to assess the influence of acute manipulation of noradrenergic signaling on goal-tracking, sign-tracking, and polydipsic drinking by an acute atomoxetine challenge following training on both autoshaping and SIP procedures (**Figure 1A**). Experiment 2 aimed to investigate, in a separate cohort (n=60), the effect of chronic atomoxetine on well-established, aDLS-dopamine habit system-dependent hyperdipsia, the same treatment as previously shown to prevent the development of hyperdipsia in highly impulsive rats (25) (**Figure 1B**). Experiment 2 also enabled us to investigate the effect of atomoxetine on the neural systems and molecular signatures of hyperdipsia via post-mortem quantitative polymerase chain reaction (qPCR) analyses (**Figure 1B**).

Behavioural training

Autoshaping was conducted in twelve dedicated MEd Associates operant boxes as described previously (69,70) and detailed in the **Supplementary Methods**. Chiefly, each autoshaping session consisted of twenty-five 8-second presentations of a retractable lever and stimulus light (compound CS) on a variable interval (VI) 90-second schedule. One food pellet was delivered into the adjacent magazine immediately following the 8-second CS presentation. Approaches to the compound CS resulted in contact with the lever (ie. CS lever presses), which had no programmed consequence but were recorded to measure sign-tracking. Goal-tracking behaviour was assessed by the number of head entries made into the food magazine during the CS presentation (ie. CS magazine entries).

SIP habituation, magazine training, and SIP training were carried out in twenty-four dedicated Med Associates boxes as described previously (29) and detailed in the **Supplementary Methods**. Briefly, during a 60-minute SIP session, food pellets were delivered on a fixed time 60 second (FT60s) schedule. A 300mL water bottle was freely accessible throughout the session, and water consumption per session was calculated in mL for each rat [(pre-session bottle weight) – (post session bottle weight), in grams].

Drugs

Atomoxetine hydrochloride (ATO, Sequoia Research Products, UK) was dissolved in sterile 0.9% saline vehicle. For the acute atomoxetine challenge in Experiment 1, each rat received intraperitoneal (i.p) administration of 0, 1.0, and 3.0mg/kg at 1 mL/kg, counterbalanced across days and rats according to a Latin Square design, and following a repeating 3-day schedule: 1) drug-free behaviour session – no injection, 2) dosing session – pre-session injection, and 3) rest – no behavioural testing or injection. In Experiment 2, following 21 days of drug-free SIP acquisition, rats were randomly assigned to receive atomoxetine (1.0 mg/kg at 1.0 mL/kg i.p (29)) or vehicle (1 mL/kg 0.9% saline, i.p) prior to each SIP session for the next 17 days. Within each drinking group (ie. HD, IHD, ILD, and LD, see below), randomly assigned treatment groups were matched for baseline pre-treatment water consumption. All injections occurred 30 minutes before behavioural testing.

Molecular analyses

Forty-five minutes after the final SIP session, rats in Experiment 2 were anaesthetized under isoflurane and decapitated. Brains were rapidly extracted and flash frozen in -40°C isopentane. 300µm thick coronal sections of the brains were µ-punched as previously described (71) and detailed in the **Supplementary Methods.** The following regions, each previously shown to be involved in compulsion or in the transition from goal-directed to habitual control over behaviour (26,38,72–79) were collected: anterior and posterior insular cortex (aIC and pIC), nucleus accumbens shell and core (NAcS and NAcC), anterior dorsolateral striatum (aDLS), posterior dorsomedial striatum (pDMS), and basolateral and central amygdala (BLA and CeA). RNA was extracted, cDNA banks were reverse transcribed, and mRNA levels were quantified in duplicate runs using qPCR as previously outlined (71) and detailed in the **Supplementary Methods**. Genes of interest and respective primers are outlined in **Supplemental Table 1**.

Data and statistical analyses

Data, presented as means ± SEM and/or individual data points, were analysed using SPSS 28.0.1 (IBM Corporation, USA). Assumptions of normality, homogeneity of variance, and sphericity were tested using Shapiro-Wilk, Levene, and Mauchley tests, respectively. In instances where normality was violated, as for a few genes in the qPCR experiment, data were log-transformed before further analysis. If sphericity was violated, the Huynh-Feldt correction

was applied. All statistical analyses were conducted in SPSS 28.0.1 (IBM Corporation, USA). Figures were generated using GraphPad Prism 10.2 (GraphPad, USA) and Adobe Illustrator (CA, USA). Network visualizations were created using Cytoscape 3.10 (Institute for Systems Biology, USA).

Data were subjected to Analyses of Variance (ANOVA) with group (ST vs GT or HD, IHD, ILD, LD) and/or treatment (veh vs ATO) as between-subject factors and time (daily sessions) as within-subject factor as detailed below. On confirmation of significant main effects, differences among individual means were further analyzed using Sidak-corrected post-hoc comparisons. Effect sizes for ANOVA effects and pairwise comparisons were reported as partial eta squared (η^2) or Cohen's d, respectively. Relationships between continuous variables were assessed using Spearman correlations as detailed below.

Behavioural classification

Baseline autoshaping and SIP behaviour was determined based on an individual's average level of responding (ie. CS lever presses, CS magazine entries, and SIP water consumption) across the last three pre-treatment sessions of either paradigm. Rats in Experiment 1 were classified as goal trackers (GTs), intermediate performers (INT) or sign trackers (STs) using k-means clustering based on baseline CS lever presses and CS magazine entries. Rats from both experiments were stratified by a quartile split of baseline SIP water consumption and classified as high (HD), high intermediate (IHD), low intermediate (ILD), and low (LD) drinkers. This approach segregates hyperdipsic rats (HD) from rats which do not develop an adjunctive coping response (LD) (24,26,29,69,80). Further, we can distinguish rats which develop adaptive low levels of adjunctive coping (ILD) from those which acquire polydipsia, yet do not exhibit extreme, compulsive hyperdipsia (IHD). The relative distribution of STs and GTs across the four SIP groups was assessed using a Chi-square test, followed by a z-test for independent proportions.

Behavioural performance and drug challenge

Longitudinal SIP and autoshaping data were analyzed using repeated measures analysis of variance (ANOVA) with session as a within-subject factor. SIP sessions were divided into equal-sized blocks of training and treatment sessions (early/late training or treatment, where applicable) to assess potential group differences across distinct epochs of the experiment. For all repeated measures analyses of SIP data, block was included as a within-subjects factor. To compare task performance between groups in Experiment 1, autoshaping cluster (GT/ST) and drinking group (HD/IHD/ILD/LD) were set as between-subject factors. Acute atomoxetine challenge data were analyzed using repeated measures ANOVA with dose (3 levels: 0,1,3mg/kg) as a within-subject factor, and autoshaping group (ST/GT) and drinking group (HD/IHD/ILD/LD) as between-subject factors. To investigate the effect of chronic atomoxetine treatment in Experiment 2, and how this may differ between rats which develop distinct coping strategies, treatment (atomoxetine/vehicle) and drinking group (HD/IHD/ILD/LD) were included as between-subject factors. Any significant main effects or interactions were further examined by Sidak-corrected pairwise comparisons between appropriate group means.

Linear regression of baseline performance was conducted as follows, for each SIP group separately, to assess the predictive relationship between autoshaping and SIP, and whether the relationship differs between drinking groups. Multivariate linear regression, with both CS lever presses and CS magazine entries as predictors, was used to assess the extent to which both autoshaping traits together predict subsequent SIP. Univariate linear regression, with either CS lever presses or CS magazine entries as the predictor and water consumption as the outcome variable, examined whether SIP drinking could be predicted by either autoshaping trait alone.

IEG hotspot analysis

To examine potential SIP-related 'hotspots' of neuronal activity, relative mRNA expression of the immediate early genes (IEGs), cFos and zif268, were assessed in 8 brain regions of interest (see "Molecular analyses") using univariate ANOVA with treatment (atomoxetine/vehicle) and compulsivity group (HD/LD) as between-subject factors. Significant main effects or interactions were followed by appropriate Sidak-corrected pairwise comparisons. IEG levels in each region of interest were also correlated with water consumption on the final SIP session using Spearman correlation to further interrogate the relationship between regional activation patterns and polydipsic coping behaviour.

NAcS transcriptional network analysis

To explore atomoxetine- and compulsivity-related transcription patterns in the NAcS, the noradrenaline-innervated accumbal subregion (24,67,68) which actuates atomoxetine's anti-impulsivity benefit (62), expression of various genes of interest (Supplementary Table 1) within were first compared between groups using a univariate ANOVA with treatment and group as between-subject effects. To explore transcriptional relationships, Spearman correlations were performed between all NAcS genes of interest for each treatment and compulsivity group separately. Resultant p-values were adjusted using the Benjamini-Hochberg correction for multiple comparisons. Correlations that survived correction were visualized using network plots, whereby each node represented a gene, and edge thickness/opacity corresponded to the correlation's Spearman Rho value. Non-significant correlations were omitted, and node size was proportional to the number of significant correlations a given target had with other genes.

Results

Experiment 1:

Behavioural classification

In the autoshaping task, distinct groups were revealed by k-means clustering based on approach tendency (Figure 1C). STs made more CS-concurrent lever presses than GTs (Figure 1D), which entered the food magazine more than the former during CS presentation (Figure 1D). Four additional groups were characterized by average water consumption during the last three SIP sessions: HD, which exhibited hyperdipsia; LD, which did not develop polydipsia for water (25,69); alongside IHD and ILD which demonstrated different degrees of adaptive coping (ie. intermediate drinking) (Figure 1E). HD rats drank more than LD rats from the third SIP session onward (Figure 1F). The difference between IHD and ILD water consumption emerged after the 15th session (Figure 1F). Rats were unevenly distributed across SIP groups and GT/ST clusters, such that rats in the IHD and ILD groups were exclusively classified as either STs or GTs, respectively (Figure 1G). There were also more GTs in the ILD group than any other drinking group (Figure 1G).

Relationship between autoshaping and SIP

We first examined whether GTs and STs differed in their tendency to develop SIP and its compulsive manifestation, hyperdipsia. GTs and STs differed in the trajectory of SIP acquisition, whereby STs drank significantly more water than GTs during early SIP sessions (d1-9) (Figure 2A), consistent with the relatively greater proportion of GTs in lower drinking groups (ILD, LD) (Figure 1G). As SIP became more established, GTs eventually reached comparable drinking levels to STs such that water consumption during later training sessions (d10-18) was no longer distinguishable between the groups (Figure 2A). We then retrospectively assessed the autoshaping profile of rats characterized by the level of polydipsia they established in SIP. Rats

that developed hyperdipsia (HD) did not differ in goal-tracking magazine entries nor sign-tracking lever presses from those which failed to acquire polydipsia whatsoever (LD) (Figure 2B,C). However, ILD rats, which developed low levels of polydipsia, made fewer sign-tracking lever presses (Figure 2D) and more goal-tracking magazine entries (Figure 2D) than both IHD and HD rats, whose polydipsic coping responses were high or excessive, respectively. This indicates that Pavlovian conditioned approach tendencies are expressed to the lowest degree in the group of rats which adopt a moderate, adaptive coping strategy. Goal-tracking and sign-tracking of adaptive ILD rats did not significantly differ from that of the LD group.

A multivariate liner regression model used to estimate the extent to which the level of adjunctive drinking could be predicted by both goal-tracking and sign-tracking did not reach significance in any drinking group (HD: $F_{2,9} = 1.980$, $p = 0.194$; IHD: $F_{2,9} = 0.383$, $p = 0.693$; ILD: $F_{2,9} = 3.052$, $p = 0.097$; LD: $F_{2,9} = 0.297$, $p = 0.769$). Simple linear regression analyses were performed to examine whether the level of drinking could be predicted by either autoshaping trait independently, indicating that goal-tracking magazine entries significantly predicted SIP drinking, but only in the ILD group (Figure 2E). In ILD rats, goal-tracking accounted for 39.3% of the variance in drinking (Figure 2E) [$R^2 = 0.393$; regression equation: (baseline ILD water consumption) = 10.032 + 0.017*(baseline CS magazine entries)]. Sign-tracking alone did not significantly predict water consumption for any drinking group (Figure 2G,H).

Acute pharmacological manipulations

Acute atomoxetine did not influence sign-tracking in any group (Figure 3A). There was a trend toward a reduction in magazine entries in GTs at the highest dose (Figure 3B), and the low dose of atomoxetine reduced magazine entries in ILD rats (Figure 3bB). The selective effect of atomoxetine on magazine entries in the ILD group appeared to be driven by the tendency of ILD rats to express high levels of goal-tracking behaviour at baseline (Figure 2D), as when baseline magazine entries were covaried in the above analysis, the effect in the ILD group was no longer significant (Figure 3B). Acute administration of atomoxetine did not significantly affect the expression of polydipsia in any group (Figure 3C).

Experiment 2

SIP acquisition

As in Experiment 1, subjects were classified as HD, IHD, ILD, or LD, based on a quartile split of baseline water consumption, to examine potential group differences in behavioural sensitivity to atomoxetine. ILD and IHD groups demonstrated comparable water drinking until the 12th SIP training session, and HD rats drank more water than LD rats from the 3rd session.

Effect of chronic atomoxetine on well-established SIP

After 21 SIP sessions, subjects were randomly assigned to receive i.p. vehicle or atomoxetine prior to each daily SIP session for 17 days, with groups matched for baseline water consumption (Figure 4A,B). Chronic atomoxetine administration selectively exacerbated hyperdipsia in HD rat across the entirety of treatment (Figure 4C), yet it did not influence drinking in LD rats (Figure 4C) or either intermediate drinking group (Figure 4D).

Molecular correlates of compulsive SIP and atomoxetine-invigorated hyperdipsia

IEG hotspot analysis:

Brain regions involved in the selective exacerbation of hyperdipsia by chronic atomoxetine were explored using an IEG (ie. cFos, zif268) hotspot analysis within eight relevant brain regions (see Methods). Atomoxetine-treated rats, irrespective of drinking group, exhibited higher cFos expression in both the aDLS and pDMS (Figure 5A), and contrastingly reduced zif268

expression in the aDLS (Figure 5B) compared to vehicle-treated controls. These findings are intruiging considering that the dorsal striatum does not receive noradrenergic innervation (67,81). The atomoxetine group appeared to demonstrate reduced cFos expression in the anterior insular cortex (aIC), yet this effect did not reach statistical significance (p=0.066) (Figure 5C). No significant main effects or interactions were observed in any other region of interest (Figure 5C,D) (all ps ≥ 0.066). Expression levels of various target genes (

IEG-behaviour correlations:

To further explore the relationship beween regional brain activation and atomoxetine-invigorated hyperdipsia, IEG mRNA levels in all regions of interest were correlated with water consumption on the final SIP session, after which brains were harvested. Levels of zif268 in the NAcS positively tracked water consumption in vehicle-treated HD rats only (rho = 0.788) (Figure 5E). In vehicle-treated rats, irrespective of drinking group, water consumption positively correlated with zif268 expression in the aDLS (rho = 0.524) (Figure 5F) and NAcC (rho = 0.596) (Figure 5G). In atomxetine-treated HDs, water consumption was negatively correlated with NAcC cFos expression (rho = -0.762) (Figure 5H), and positively correlated with CeA cFos expression (rho = 0.690, p = 0.058) (Figure 5I). When HDs in either treatment group were pooled, the correlation between CeA cFos and water consumption did not reach significance (rho = 0.488, p = 0.065). Neither NAcC or CeA cFos levels significantly correlated with water consumption in LDs nor vehicle-treated animals (Figure 5J,K). No other significant correlations were observed (all ps ≥ 0.073).

NAcS transcriptional analysis:

Within the NAcS, no significant differences in mRNA levels were observed between drinking and treatment groups for any individual gene of interest (all Fs ≤ 2.304; all ps ≥ 0.146) (Supplementary Figure 1A,B). To explore compulsivity- and treatment-related transcriptional signatures in the NAcS, intra-NAcS mRNA levels for various genes of interest were correlated for HD and LD in either treatment condition. The correlational transcriptional profiles of vehicle-treated LD and HD rats were distinct, particularly regarding the prevalence or absence, respectively, of catecholamine-catecholamine and catecholamine-IEG correlations (Figure 5L,M). Vehicle-treated HD rats also exhibited fewer overall correlations (Figure 5M) compared to the highly correlated transcription of LD controls (Figure 5L). Notably, noradrenergic receptor (NAr) transcription was largely dissociated from IEG expression in HD vehicle rats (Figure 5M), while LD controls exhibited numerous strong NAr-IEG correlations (Figure 5L). Chronic atomoxetine treatment resulted in unique transcriptional signatures in the NAcS of LD and HD rats (Figure 5N,O), including the maintenance of a dopamine-kappa opioid system correlation in LD rats (Figure 5N), and the emergence of dopamine receptor-cFos expression relationships in HD rats (Figure 5O). Spearman rho values are presented in Supplementary Table 2.

Discussion

In the current set of experiments, we showed that sign-tracking relates to expedited acquisition of adjunctive coping at the population level, and in a subgroup of rats which adopt an adaptive coping strategy, goal-tracking positively predicts SIP drinking. In contrast to the protective effect of atomoxetine against the *development* of hyperdipsia (29), we demonstrated that chronic atomoxetine *exacerbates* hyperdipsia once it is well-established, yet has negligible acute effects on well-established compulsive coping. Further, we demonstrated that chronic atomoxetine increases cFos expression in both the aDLS and pDMS, while concomitantly reducing expression of the plasticity marker zif268 (82,83) in the aDLS. We also revealed that zif268 levels in the aDLS and NAcC are positively associated with polydipsia in controls, while in atomoxetine-treated compulsive HD rats, cFos expression in the NAcC and CeA negatively and

positively correlate with water intake, respectively. Additionally, we revealed reduced transcriptional coherence between catecholamine markers in the NAcS of vehicle-treated HDs, and an emergent dopamine receptor-IEG expression correlation in the NAcS of atomoxetine-treated HDs.

The acquisition-specific difference in polydipsia between ST and GT is broadly consistent with the neurobiological mechanisms across SIP training, whereby accumbal catecholamines underpin both the expression of sign-tracking (59–61) and the early acquisition of polydipsia (35,37,84), the training epoch during which STs and GTs differ in the current study. The groups no longer differ once SIP becomes more well-established and is under control of not accumbal catecholamines, but the aDLS habit system instead (25). Given its antecedence to and association with drinking-related anxiolysis (21,22,24), the SIP chamber itself could acquire incentive salience as an occasion setter (85) through negative reinforcement (24,25). Thus, exposure to the chamber could invigorate water drinking via Pavlovian-to-instrumental transfer (PIT) (85–87). Since STs more readily form Pavlovian associations than GTs (88), PIT may contribute to their expedited development of polydipsia early on. The difference between STs and GTs may disappear with further training because GTs eventually form the same Pavlovian associations as STs, albeit at a slower rate, consistent with evidence that GTs eventually establish some degree of sign-tracking with extended training on the autoshaping paradigm (89).

We also showed that ILD rats demonstrate more goal-tracking behaviour, and goal-tracking positively correlates with SIP drinking in these individuals. ILD rats likely exemplify adaptive copers, which adopt a controlled adjunctive strategy to reduce stress without incurring negative metabolic effects associated with excessive water consumption. Contrastingly, in groups which develop more extreme polydipsia (ie. IHD) or compulsive hyperdipsia (ie. HD), or fail to acquire a coping response altogether (ie. LD), goal-tracking did not relate to SIP drinking. Thus, goal-tracking may predict the predilection toward adaptive coping strategies, consistent with theories of goal-tracking as a SUD-resilient endophenotype (90–92).

Here, acute atomoxetine did not influence sign-tracking, which conflicts with a prior report that atomoxetine reduces sign-tracking in STs (93). Notable methodological differences in the way in which GT and ST groups are defined likely account for this discrepancy, as the previous study used a tripartite Pavlovian conditioned approach index to classify rats (93), while we employed a k-means clustering algorithm. Our approach may engender a more conservative ST criterion, given that STs make up a smaller proportion of our experimental cohort (22% vs 40%) and our STs made relatively more lever presses at vehicle (average ~90 vs ~65) compared to those reported in the aforementioned paper (93). Thus, our STs may exhibit more extreme Pavlovian approach tendencies that are less pervious to acute pharmacological manipulation. Our atomoxetine-induced reduction in goal-tracking s selectively in GTs is generally consistent with Holden et al.'s work (93) whereby atomoxetine appears to more markedly influence goal-tracking in GTs, although this interaction did not each statistical significance (93). Our acute pharmacological manipulations, however, failed to influence well-established SIP, highlighting the possible necessity of chronic treatment, and the consequent neuroplastic adaptations, to sway well-established polydipsia.

Our chronic pharmacological data reveal that repeated atomoxetine treatment *exacerbates* well-established hyperdipsia, which is in stark contrast to our lab's previous findings whereby atomoxetine *prevents* the development of hyperdipsia in vulnerable rats (29). These opposing results suggest that SIP-related neural plasticity (24) alters the mechanisms through which noradrenaline signaling controls coping behaviour between emergent polydipsia in vulnerable individuals and its eventual compulsive manifestation. One possible mechanism could relate to the LC-noradrenaline system, given that the plasticity-related gene Arc is downregulated in a

subset of LC neurons in HD rats (24). Therefore, following extended SIP, the LC-noradrenaline system of HDs may be relatively aplastic, and thus incapable of orchestrating the treatment-related neuroplastic adaptations that underpin atomoxetine-induced resilience against hyperdipsia (29), allowing other pro-compulsive mechanisms (ie. aDLS recruitment, discussed below) to dominate control over behaviour. Furthermore, the fact that atomoxetine selectively influences SIP in HD rats, regardless of when treatment occurs (29), suggests an underlying difference in the catecholaminergic system of HD individuals which renders them particularly sensitive to perturbations of noradrenaline signaling (29). Such a difference in baseline function and/or sensitivity of the noradrenaline system likely contributes to the vulnerability to develop compulsivity in HD rats.

Indeed, our molecular data expose compulsion-related alterations in the NAcS catecholamine system. Vehicle-treated HDs, compared to LD vehicle controls, exhibit a transcriptional segregation between the noradrenaline and dopamine system, generally consistent with the catecholaminergic dysfunction purported to underly both compulsive coping (24,66) and compulsion vulnerability traits like impulsivity (29,94–96). The disrupted NAr-IEG relationship seen in HD vehicle controls, in contrast to the multiple strong correlations between NArs and IEGs in vehicle-treated LDs, could also be indicative of noradrenergic dysfunction within the NAcS of HDs, as NAr transcription is dissociated from NAcS activity. Atomoxetine evokes a similar disruption between NAr and IEG transcription, regardless of whether rats are HD or LD, suggesting that chronic pharmacological alteration of noradrenergic signaling can influence the relationship between NAr expression and regional activation and plasticity in the NAcS (82,97). Further, atomoxetine-exacerbated hyperdipsia was uniquely accompanied by correlated NAcS dopamine receptor and IEG transcription. Such a relationship between the expression of dopamine receptors and NAcS activity markers (98) could be reminiscent of the accumbal dopamine-dependent activity that underlies early SIP acquisition (35,37,84). If the mesolimbic catecholamine system of HDs is re-engaged by chronic atomoxetine, which remains to be empirically confirmed, this could further intensify well-established drinking via similar processes which drive early SIP acquisition (35,37,84). Notably, atomoxetine-treated LDs maintain a significant correlation between expression of kappa-prodynorphin markers and dopamine receptors. Such a relationship may be indicative of a stress-related negative affective state (99–101) which persists despite atomoxetine treatment, consistent with the failure to develop anxiolytic adjunctive coping in LDs (24). It is important to emphasize the exploratory nature of these correlational network analyses, and thus any speculations arising from these correlations remain to be causally investigated. Nonetheless, our IEG hotspot analyses provide important mechanistic insight into the effect of atomoxetine on well-established compulsivity.

As indicated by cFos expression (97), atomoxetine treatment led to greater SIP-related functional activation of both the aDLS and pDMS, which do not receive noradrenergic innervation (67,81). This suggests indirect recruitment of the dorsal striatum, potentially by the NAcS via spiraling striato-nigro-striatal connections (38,102). Such coincident activation of the aDLS-dependent habit system and pDMS-dependent goal-directed system may introduce conflict between goal-directed and habitual control over behaviour. Given that well-established hyperdipsia is under control of the aDLS-dependent habit system (25), the increase in aDLS activation likely dominates over behaviour at this stage, leading to an exacerbation of aDLS-dependent hyperdipsia*. The atomoxetine-induced increase in pDMS activation could likewise drive the exacerbation of hyperdipsia, given that pDMS-dependent goal-directed processes underly the initial development of a behaviour prior to its habitual manifestation* (73)*, thus precipitating a further 'acquisition' of goal-directed anxiolytic drinking, on top of an already well-established hyperdipsic tendency.* Concomitantly, atomoxetine treatment decreased aDLS zif268 expression, signifying reduced activity-related plasticity in the aDLS habit system (82,83). A diminished capacity for plasticity could prevent disengagement of the aDLS-dependent habit

system, leading to the persistence, or even exacerbation, of a previously established compulsive coping behaviour (103). Atomoxetine-induced alterations in aDLS activation likely has no behavioural effect in intermediate or low drinkers given that their drinking behaviour is not compulsive and thus not under aDLS control (25). Although sub-significant in our cohort, the trend toward a reduction in aIC cFos expression following atomoxetine could indicate an impairment in interoceptive processing, which, considering that the aIC causally influences to the vulnerability to develop compulsive hyperdipsia (26), could partially contribute to the atomoxetine-induced exacerbation of hyperdipsia in compulsive rats.

Further, we observed numerous correlations between water consumption and zif268 in vehicle-treated rats only, suggesting that chronic atomoxetine may disrupt the relationship between adjunctive coping and activity-related plasticity within SIP-relevant brain regions. Our NAcS zif268 correlations indicate that heightened plasticity within the NAcS corresponds to a greater degree of hyperdipsia, at least in HDs which have not undergone chronic noradrenergic manipulation. Elevated NAcS plasticity, coupled with diminished plasticity within the noradrenergic LC (24) which innervates the NAcS (24,67,68), could create an imbalance between pre- and post-synaptic integration within the noradrenaline system. This could facilitate the development of aDLS-dependent (25) compulsive coping strategies via the NAcS's functional interactions with the NAcC- a principal regulator of goal-directed and habit system recruitment (104–106). The positive relationship between drinking and zif268 expression in the aDLS and NAcC suggests that, in a non-pharmacologically altered state and irrespective of whether drinking is adaptive or compulsive, activity-related plasticity of the aDLS and NAcC scales with water intake. This is generally consistent with the functional inter-striatal shifts which occur as compulsion develops (25), whereby heightened plasticity within critical striatal subregions could facilitate the transition toward aDLS-dependent hyperdipsia, while reduced plasticity may preclude such a functional transition.

Multiple cFos-behaviour correlations also emerge selectively in atomoxetine-treated HD rats. The negative association between NAcC cFos and atomoxetine-invigorated hyperdipsia indicates that the degree to which the NAcC is disengaged tracks the loss of control over coping, implicating the NAcC as a potentially protective mediator against the pro-compulsive effects of atomoxetine on well-established hyperdipsia. This interpretation is in line with the role of the NAcC in directing the relative engagement of habit vs goal-directed control over behaviour (104–106), as a loss of proper NAcC mediation could lead to loss of goal-directed control and a devolution toward further habitual domination over coping behaviour. Why this negative correlation arises only in atomoxetine-treated HDs, in an accumbal subregion which does not receive notable noradrenergic innervation, is unclear, yet could suggest an indirect disengagement of the NAcC via functional NAcS-NAcC interactions (107).

Moreover, as behaviour becomes habitual and aDLS-dependent, at least in the case of well-established drug seeking, amygdalar control over behaviour shifts from the BLA to the CeA (74). Assuming such a shift also accompanies the development of compulsive aDLS-dependent hyperdipsia, greater CeA engagement should track the degree of hyperdipsia- a speculation which is supported by the positive correlation between CeA cFos and drinking currently observed in our atomoxetine-treated HDs. It is worth noting that this positive relationship between CeA activation and SIP drinking also appears in vehicle-treated HD rats, yet does not reach significance, potentially due to the smaller n in the vehicle group (n=7).

Overall, these data reveal a novel relationship between goal-tracking and adaptive coping, and further elucidate noradrenergic contributions to compulsive coping, highlighting the stark difference between the protective vs. pro-compulsive influence of atomoxetine on early vs. well-established hyperdipsia, respectively. Additionally, we describe a putative molecular signature of compulsivity, involving an altered transcriptional profile in the NAcS catecholamine system, and

a mechanism through which atomoxetine may indirectly re-engage the central amygdala and dorsal striatal habit and goal-directed systems to exacerbate well-established hyperdipsia. The precise circuits through which noradrenaline acts to recruit these processes, and the temporal nature of the shift in noradrenergic control over coping behaviour, have yet to be defined and are fruitful questions for future research to explore.

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Figure legends

Figure 1. Timeline of the experiments, and characterization individual differences in autoshaping and polydipsic coping behaviour.

A) Rats in Experiment 1 were allowed 7 days of habituation after arrival at the facility, prior to being food restricted to 85-90% of their theoretical free-feeding weight. Across the three consecutive days prior to autoshaping training, rats underwent habituation to sugar pellets, habituation to the autoshaping chamber, and basic magazine training. Next, rats were trained on the autoshaping paradigm (AS) for five acquisition sessions, after which the atomoxetine challenge began according to a counterbalanced Latin square design, wherein each dosing day was followed by two days of injection-free baseline autoshaping. Following the first autoshaping-concurrent Latin square, rats were further food restricted to 80-85% of their theoretical free-feeding weight, and habituated to the SIP chamber and the fixed time 60 second (FT-60) food pellet delivery schedule. SIP training was then performed for 18 60-min sessions. The second atomoxetine challenge occurred according to the same dosing and drug-free baseline schedule as for the previous Latin square. **B)** In Experiment 2, following a 1 week acclimatization period, rats were food restricted to 80-85% of theoretical free-feeding weight. During the last two days of food restriction, rats underwent food pellet habituation, SIP box habituation and magazine training as in Experiment 1. Rats were then exposed to the SIP procedure for 21 days, during which high, high intermediate, low intermediate (ie. adaptive) and low drinkers were identified. Equal numbers of rats of each drinking group then received intraperitoneal (i.p.) injections of atomoxetine (1mg/kg) or vehicle 30 mins before each daily SIP session for 17 days. Forty-five minutes after the final SIP session, rats were sacrificed and their brains were harvested and flash frozen for subsequent qPCR analyses. **C)** A k-means clustering algorithm was used to categorize rats as sign trackers (STs), goal trackers (GTs), or intermediate performers (INT) based on average lever presses and magazine entries made during the CS across the last three baseline autoshaping training sessions. The “X”s represents cluster centers. **D)** Both traits were developed across the five autoshaping sessions (CS lever presses- session: $F_{4,180} = 33.161$, $p < 0.001$, $\eta^2 = 0.424$; session 1 vs 5: Sidak $p < 0.001$, $d = 1.176$; CS magazine entries- session: $F_{4,180} = 41.071$, $p < 0.001$, $\eta^2 = 0.483$; session 1 vs 5: Sidak $p < 0.001$, $d = 1.334$). As expected, STs and GTs demonstrated greater responding either on the lever or at the food magazine, respectively, during the CS presentation (CS lever presses- AS session x AS group: $F_{8,180} = 17.240$, $p < 0.001$, $\eta^2 = 0.434$; GTs vs STs- session 1: Sidak $p = 0.003$, $d = 0.504$; all other sessions: Sidak ps < 0.001; CS magazine entries- AS session x AS group: $F_{8,180} = 23.766$, $p < 0.001$, $\eta^2 = 0.514$; GTs vs STs- session 1: Sidak $p = 0.094$; session 2: Sidak $p < 0.001$, $d = 1.165$; all other sessions: Sidak ps < 0.001). **E)** Rats were defined as high (HD), high intermediate (IHD), low intermediate (ILD), or low (LD) drinkers based on a quartile split of baseline water consumption (ie. average across the last 3 days of SIP training). **F)** Adjunctive water drinking was acquired by the third day of SIP training at the level of the population (session: $F_{17,784} = 55.614$, $p < 0.001$, $\eta^2 = 0.558$; session 1 vs 2: Sidak $p = 0.238$, session 1 vs 3: Sidak $p < 0.001$, $d = 1.221$; session 1 vs all other sessions: all ps ≤ 0.001). Differences between drinking groups emerged at various points in acquisition, such that by the end of SIP training, groups exhibited distinct levels of water consumption across the last three baseline (BL) SIP sessions (SIP session x drinking group: $F_{51,782} = 3.402$, $p < 0.001$, $\eta^2 = 0.188$; HD vs LD- sessions 1-2: Sidak ps ≥ 0.770; sessions 3-18: Sidak ps ≤ 0.042, ds ≥ 0.408; IHD vs ILD- sessions 1-14: Sidak ps ≥ 0.152; sessions 15-18 ps ≤ 0.037, ds ≥ 0.393). **G)** Across drinking groups, there was a disproportionate representation of GTs and STs ($\chi^2_{3,n=19} = 10.795$, $p = 0.013$), such that there were fewer STs vs GTs in the ILD group (ILD- GT vs ST: $z = -3.055$, $p = 0.002$), and more STs than GTs in the IHD group (IHD- ST vs GT: $z = -1.920$, $p = 0.055$). There were also significantly more GTs in the ILD group versus any other drinking group (STs- ILD vs

HD: z = -2.582, p = 0.010; ILD vs IHD: z = -3.000, p = 0.003; ILD vs LD: z = -2.070, p = 0.038; all other |zs| ≤ 1.920, ps ≥ 0.055). Data are presented as A) individual datapoints representing each subject, B-D) group means ± SEM, or E) number of subjects per group. ******* indicates significant Sidak-corrected group differences between all groups on the last three training sessions. ***** and line: p < 0.056 according to a z-test of independent proportions.

Figure 2. Sign-tracking is associated with faster development of SIP, while goal-tracking predicts controlled adjunctive coping.

A) STs acquired polydipsic coping under SIP earlier than GTs (block x SIP session x AS group: $F_{8,136}$ = 2.722, p = 0.008, η^2 = 0.138; early block- SIP session x AS group: $F_{8,136}$ = 2.375, p = 0.020 η^2 = 0.123; AS group: $F_{1,17}$ = 5.127, p = 0.037, η^2 = 0.232; GTs vs STs- sessions 1,2,7,8: Sidak ps ≤ 0.033, ds ≥ 0.335; sessions 3-6: Sidak ps ≥ 0.057, ds ≤ 0.294). However, as SIP training progressed, GTs reached comparable levels of SIP drinking to STs (late block SIP session x AS group: $F_{8,136}$ = 1.330, p = 0.234; AS group: $F_{1,17}$ = 1.830, p = 0.194). **B-C)** Autoshaping behaviour did not differ between rats that developed hyperdipsia (HD) and those which did not develop adjunctive coping (LD) (session x drinking group- CS lever presses: $F_{12,176}$ = 2.949, p = 0.007, η^2 = 0.167; CS magazine entries: $F_{12,176}$ = 3.968, p < 0.001, η^2 = 0.213; HD vs LD- CS lever presses: all sessions Sidak ps ≥ 0.485; CS magazine entries: all sessions Sidak ps ≥ 0.413). However, goal-tracking and sign-tracking differed between rats that expressed more intermediate levels of polydipsia, whereby **D)** adaptive low intermediated drinkers (ILD) exhibited a more goal-tracker-like phenotype, making fewer CS lever presses (ILD vs IHD- sessions 3-5: Sidak ps ≤ 0.027, ds ≥ 0.431; sessions 1-2: Sidak ps ≥ 0.515; ILD vs HD- sessions 2-5: Sidak ps ≤ 0.048, ds ≥ 0.400; session 1: Sidak p = 0.089) and more CS magazine entries (ILD vs IHD- sessions 3-5: Sidak ps ≤ 0.030, ds ≥ 0.426; session 1-2: Sidak ps ≥ 0.598; ILD vs HD- sessions 3-5: Sidak ps ≤ 0.040, ds ≥ 0.410; sessions 1-2: Sidak ps ≥ 0.080) than both high intermediate drinkers (IHD) and compulsive HD rats. Autoshaping behaviour between ILD and LD groups did not differ (ILD vs LD- CS lever presses: all sessions Sidak ps ≥ 0.146; CS magazine entries: all sessions Sidak ps ≥ 0.110). **E-F)** Simple linear regression indicated that goal tracking magazine entries predicted SIP water consumption, yet only in the ILD group (ILD: $F_{1,10}$ = 6.462, p = 0.029; HD: $F_{1,10}$ = 2.943, p = 0.117; IHD: $F_{1,10}$ = 0.371, p = 0.556; LD: $F_{1,10}$ = 0.040, p = 0.845) [R2 = 0.393; regression equation: average ILD water consumption = 10.032 + 0.017*(average CS magazine entries)]. **G)** Sign-tracking lever presses, however, did not predict SIP water consumption in any group (HD: $F_{1,10}$ = 0.142, p = 0.714; IHD: $F_{1,10}$ = 0.047, p = 0.832; ILD: $F_{1,10}$ = 1.733, p = 0.217; LD: $F_{1,10}$ = 0.430, p = 0.527). For A-D), data are presented as group means ± SEM. Individual values with the linear regression line ± a 95% confidence interval are plotted in E-G). # p < 0.05 Sidak-corrected group comparison for the indicated block. * and line: p < 0.05 Sidak-corrected group comparison across the indicated sessions. * alone: significant linear regression.

Figure 3. *Acute atomoxetine administration has negligible effects on autoshaping and schedule-induced polydipsia.*

A) Atomoxetine did not influence sign-tracking in any group (CS lever presses: all Fs ≤ 0.237, ps ≥ 0.880), yet, at the high dose, atomoxetine produced a trend-level reduction in goal-tracking in GTs only (CS magazine entries- dose x AS group: $F_{4,76}$ = 3.160, p = 0.019, η^2 = 0.143; GTs- 3.0 vs vehicle: Sidak p = 0.055, d = 0.354; all other Sidak ps ≥ 0.090). **B)** The low dose of atomoxetine reduced goal-tracking behaviour selectively in ILD rats (dose x drinking group: $F_{6,76}$ = 2.297, p = 0.043, η^2 = 0.154; ILD- 1.0 vs vehicle: Sidak p = 0.002, d = 0.536), yet this effect appeared to be driven by the high rate of CS magazine responding in the ILD group, as when baseline CS magazine entries were covaried, this was no longer statistically significant (dose x drinking group: $F_{6,74}$ = 2.240, p = 0.060; ILD- 1.0 vs vehicle: Sidak p = 0.372). **C)** Acute

atomoxetine did not affect well-established SIP behaviour in any group (dose: $F_{2,76} = 2.911$, $p = 0.061$, $\eta^2 = 0.071$; all dose x group interactions: Fs ≤ 2.301, ps ≥ 0.096; all rats- 1.0 vs vehicle: Sidak p = 0.998; 3.0 vs vehicle: Sidak p = 0.092). Data are presented as group means ± SEM. **&** Sidak $p < 0.056$ compared to vehicle. **%** Sidak $p < 0.05$ versus vehicle, but $p > 0.05$ with baseline CS magazine entries as a covariate.

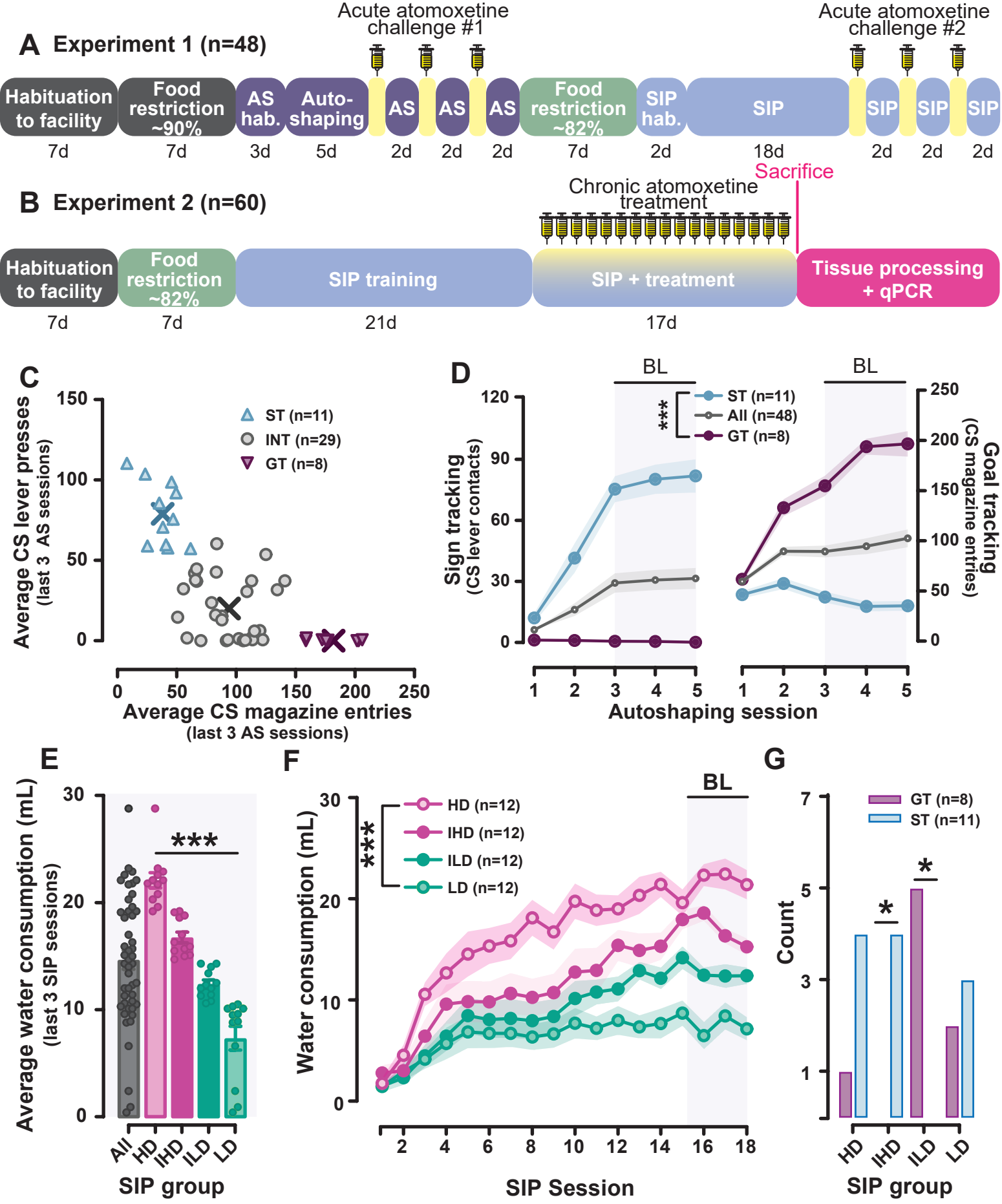
Figure 4. Chronic atomoxetine treatment selectively exacerbates well-established hyperdipsia in compulsive, high-drinking, rats and results in differential recruitment of compulsion-relevant circuitry.

A-B) Distinct SIP phenotypes emerge across 21 days of SIP training (session x drinking group: $F_{60,980} = 4.952$, $p < 0.001$, $\eta^2 = 0.408$; HD vs LD- sessions 1: p = 0.066; sessions 2-12: ps ≤ 0.054, ds ≥ 0.392; IHD vs ILD- sessions 1-11: Sidak ps ≥ 0.138; sessions 12-21: ps ≤ 0.044, ds ≥ 0.403). Groups that went on to receive chronic atomoxetine or vehicle treatment were randomly assigned and matched for baseline water consumption (treatment group: $F_{1,50} = 0.432$, $p = 0.519$; treatment group x drinking group: $F_{3,50} = 0.754$, $p = 0.525$; atomoxetine vs vehicle: all groups Sidak ps ≥ 0.131). **C)** Excessive drinking of HD rats was exacerbated by chronic atomoxetine throughout the entirety of treatment (block x treatment x drinking group: $F_{9,147} = 3.656$, $p = 0.003$, $\eta^2 = 0.183$; HD: atomoxetine vs vehicle- early treatment block: Sidak p = 0.001, d = 0.500; late treatment block: Sidak p = 0.012), d = 0.380. **C-D)** However, chronic atomoxetine treatment did not influence water consumption in IHD, LD or ILD groups (atomoxetine vs vehicle- early treatment block: all Sidak ps ≥ 0.117; late treatment block: all Sidak ps ≥ 0.350). Data are presented as group means ± SEM, with data from individual subjects shown in A and B. Blue shaded regions in A-D indicate baseline data ie. the last three drug-free sessions prior to the commencement of treatment. Treatment sessions are indicated by grey rectangles in C and D. ***p** < 0.05 Sidak-corrected group comparison for the indicated groups.

Figure 5. Chronic atomoxetine alters SIP-related cellular activity and plasticity within compulsivity-related brain regions and differentially influences the transcriptional NAcS landscape of compulsive, high-drinking and low-drinking rats.

A) Compared to vehicle controls, atomoxetine-treated rats exhibited greater mRNA levels of the activity-related marker cFos in both the aDLS (treatment: $F_{1,27} = 6.881$, $p = 0.014$, $\eta^2 = 0.203$) and pDMS (treatment: $F_{1,27} = 4.913$, $p = 0.035$, $\eta^2 = 0.154$, and **B)** a concomitant reduction in expression of the plasticity marker zif268 selectively in the aDLS (treatment: $F_{1,27} = 5.347$, $p = 0.029$, $\eta^2 = 0.165$). **C-D)** IEG levels in the insula cortex, nucleus accumbens, and amygdala were comparable between treatment groups (all Fs ≤ 3.668, ps ≥ 0.066). High and low drinkers did not differ in IEG expression across all regions of interest (all Fs ≤ 1.060, ps ≥ 0.312) (data not shown). **E)** NAcS zif268 levels positively correlated with water drinking, yet only in vehicle-treated HD rats (rho = 0.788, p = 0.035; all other groups: all ps ≥ 0.643). In vehicle-treated rats, zif268 expression in the **F)** aDLS (rho = 0.524, p = 0.045) and **G)** NAcC (rho = 0.596, p = 0.019) positively correlated with SIP water consumption. Selectively in atomoxetine-treated HDs, cFos levels in the **H)** NAcC negatively related to SIP drinking (rho = -0.762, p = 0.028), while **I)** CeA cFos levels positively correlated with water consumption (rho = 0.690, p = 0.058). **J, K)** No significant cFos-behaviour correlations were observed in vehicle-treated HDs, nor LDs (all ps ≥ 0.094). In the NAcS, expression of IEGs and various target genes of the noradrenaline, dopamine, endogenous opioid systems were differentially correlated in **L)** vehicle-treated rats that failed to acquire polydipsia (LD) versus **M)** those which developed hyperdipsia (HD). Chronic atomoxetine treatment also resulted in divergent transcriptional patterns in **N)** LD and **O)** HD rats. For K-N), node size represents the number of significant correlations a given gene

has with other target genes, as an index of its weight in the transcriptional network. Edge width and opacity are proportional to the Spearman rho value between two nodes, and only correlations which remained significant after Benjamini-Hochberg correction are displayed. Node colour indicates the gene category: blue = noradrenaline (receptors and transporter), green = dopamine receptors, purple = kappa endogenous opioid system (peptide and receptor), yellow = IEG, orange = plasticity-related IEG. *** and line**: $p < 0.05$ Sidak-corrected comparison. *** alone:** $p < 0.059$ Spearman rank correlation.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Acute atomoxetine challenge #2
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B Experiment 2 (n=60)
Chronic atomoxetine treatment
Sacrifice
Habituation to facility
Food restriction ~82%
SIP training
SIP + treatment
Tissue processing + qPCR
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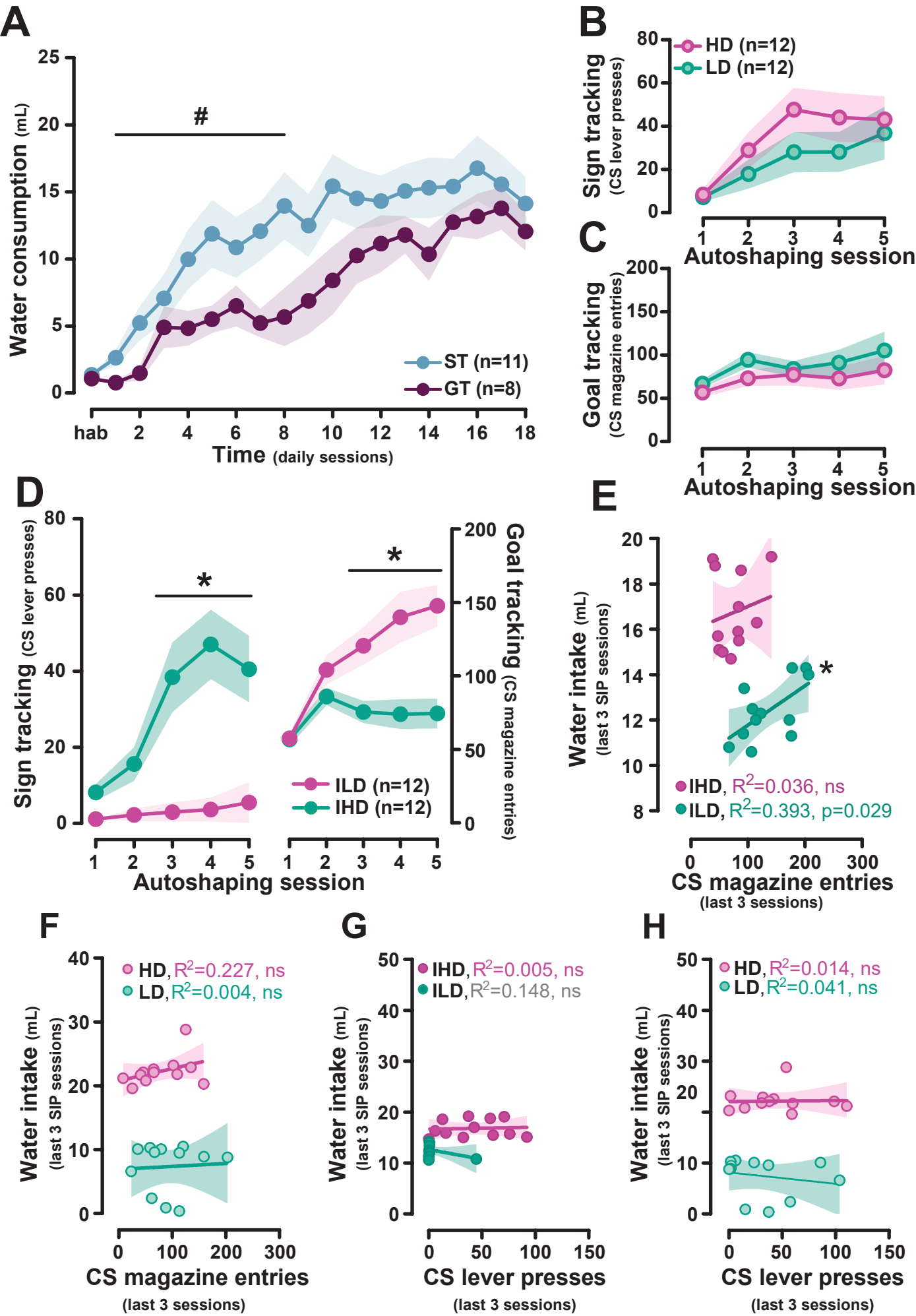
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LD, R2=0.004, ns
CS magazine entries (last 3 sessions)
G
Water intake (mL) (last 3 SIP sessions)
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ILD, R2=0.148, ns
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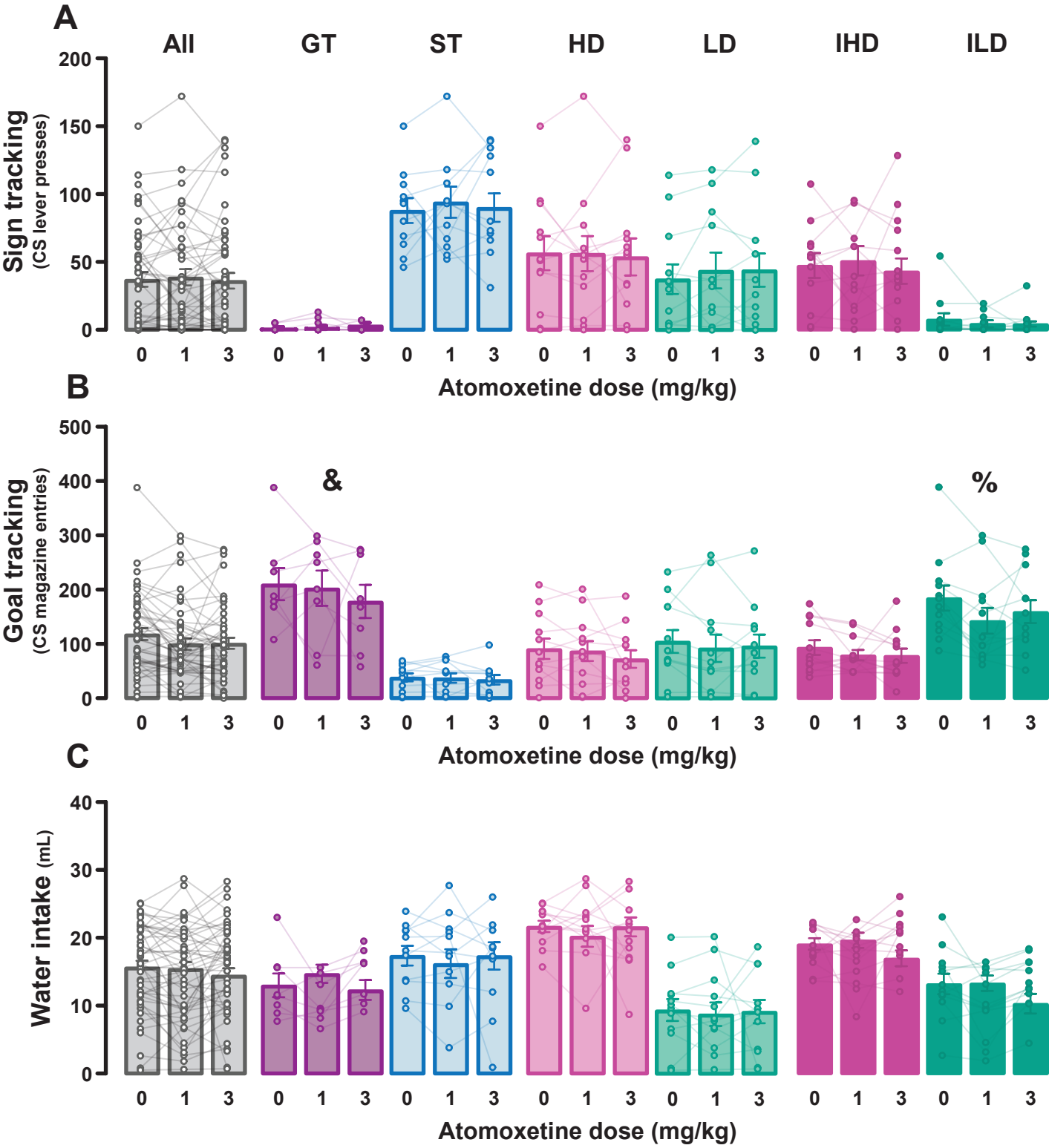
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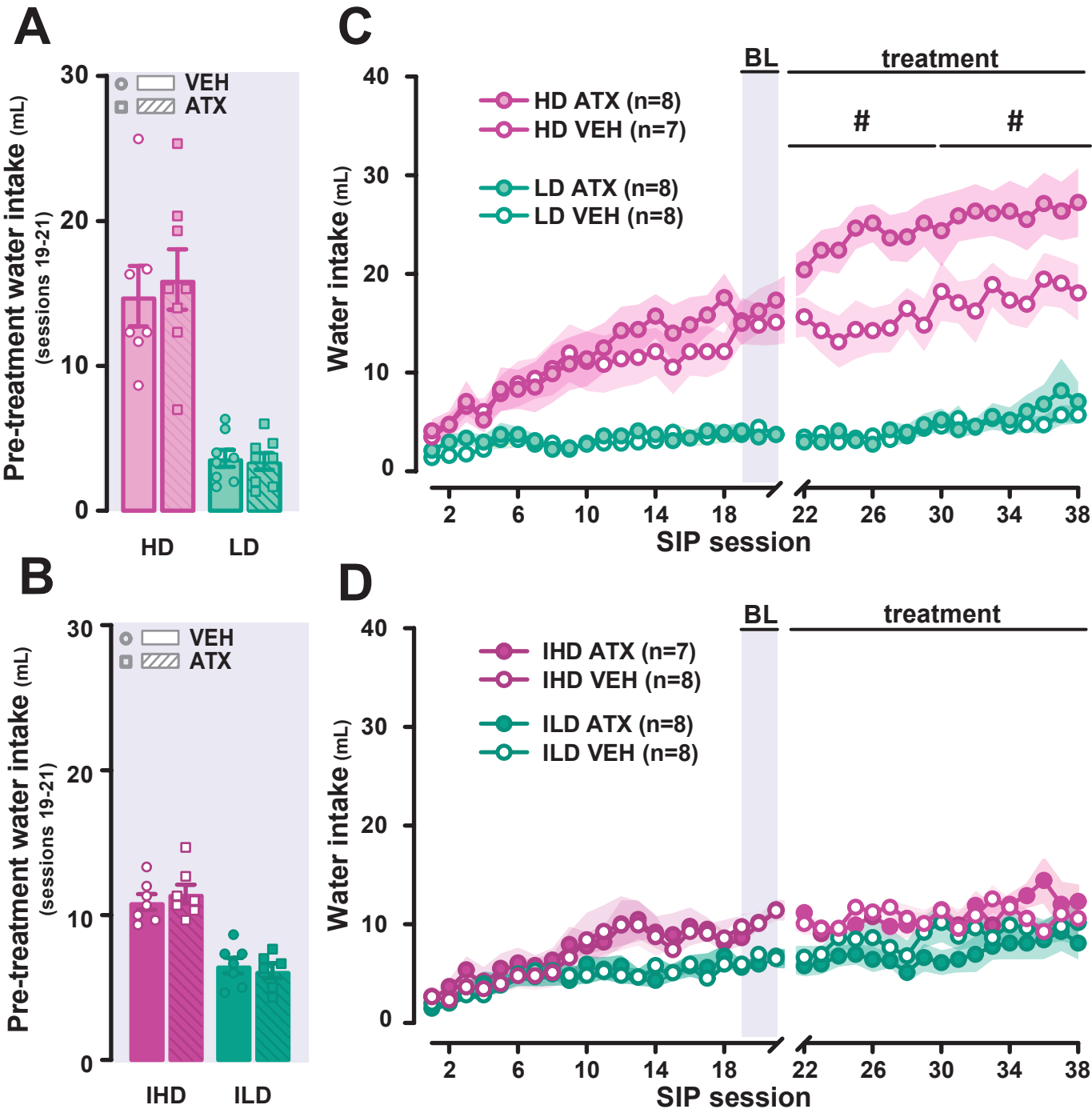
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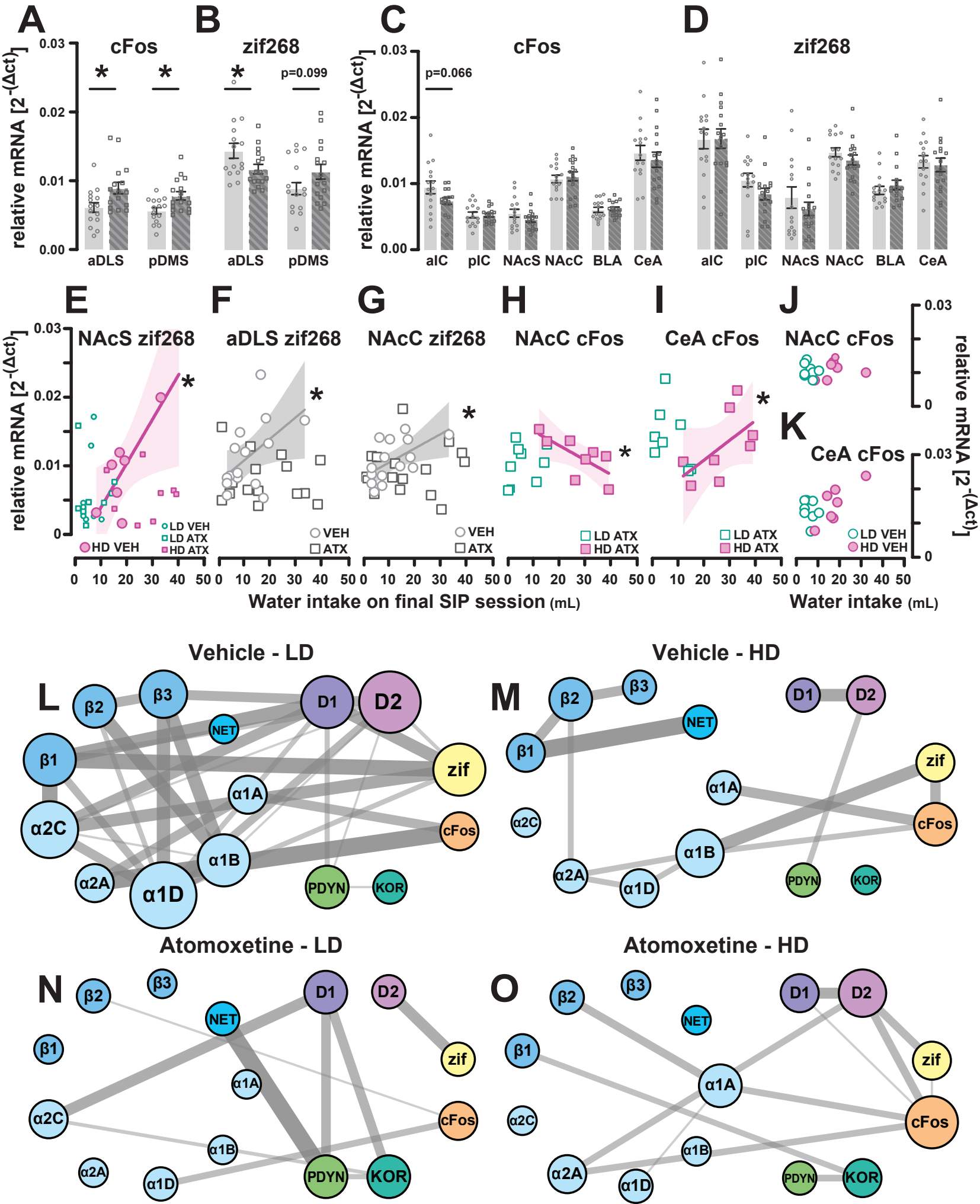
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